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THE RED STAIN IN THE WOOD OF BOXELDER[1]

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INTRODUCTION

The red stain (pl. 2) so commonly met with in the wood of living oxelder trees (*Acer negundo* L., syn. *Negundo aceroides* Moench.) has most come to be recognized as a character in the identification of this ecies. Few have stopped to consider the cause of such vivid coloring, suming in many cases that it was a normal character of the wood. uring the years of 1921 and 1922 considerable attention was drawn to is wood and to the stain which characterizes it, in efforts to discover e cause of the stain and to find means of preventing it. The vivid colring is often attractive, yet due to its irregular distribution in the heartood and its presence, at times, in the sapwood and its less attractive ades and associated colorings, the wood so stained is often found bjectionable. The wood of boxelder is used to a considerable extent r certain classes of furniture, interior finish, woodenware, cooperage, d paper pulp. In such cases the clear, creamy white color of normal ood is preferred. Therefore the disease was considered to be of sufficent economic importance to warrant an investigation of the red stain.

THE DISEASE

HISTORY

The earliest and possibly the only reference to the red stain in boxlder, in so far as the writer could determine, was published in Germany n 1880 by Eidam,[4] who states that undoubtedly some unknown ungus is responsible for the stain. He notes that it is a very characeristic stain and that it can not be confused with the discolorations proluced in coniferous wood by *Trametes pini* and *Fomes annosus* (*Trametes adiciperda*).

The writer's attention was first called to this vivid stain in November, 920, when samples of boxelder from a Tennessee lumber company were eceived for examination. Microscopical examination disclosed the yaline to slightly colored hyphae of an unknown fungus within the cells f the red-stained areas. Cultures on malt agar, made by using fragnents of the red to pink colored wood, showed a white fungous growth ttended by a pink discoloration of the agar, after incubation for seven days. In some of the tubes the white aerial mycelium seemed to dis-

[1] Accepted for publication June 25, 1923.

[2] The writer is greatly indebted to Dr. C. D. Sherbakoff for naming the fungus discussed in this paper nd for furnishing a description of it with a text figure and colored plate.

[3] In cooperation with the Forest Products Laboratory, United States Forest Service, Madison, Wis.

[4] EIDAM, E. BLAUGRÜN GEFÄRBTES HOLZ VON BIRKEN UND BUCHEN UND BLUT-BIS CARMINROTH GEFÄRBTES VON ACER NEGUNDO. *In* Jahresber. Schles. Gesell. Vaterland Cult., Jahrg. 58 (1880), p. 188-189. 1881.

appear after twenty to twenty-five days and a faintly purplish, slimy layer appeared on the agar slant. Following this, a network of jelly-like substance formed on the sides of the tubes above the agar surface. Examination disclosed this growth to be the plasmodial strands of a Myxomycete which apparently had fed upon the hyphae in the cultures. At the end of thirty days no visible signs of hyphae were apparent. Three of the tubes continued to develop normal mycelial growth, and at the end of eight to ten days a brilliant carmine stain appeared on and slightly below the surface of the agar. A subsequent study of the spore forms showed this organism to be a species of Fusarium.

Following shortly on these observations the author had occasion to study several freshly felled boxelders on the campus of the University of Wisconsin. All of these trees showed an abundance of the red stain extending from the roots to the smaller branches. It is most commonly found in the heartwood, but in many cases the discolored zone appeared in the inner sapwood, and isolated patches and irregular areas of color appeared in the sapwood nearer the bark (pl. 2). Cultures were secured from samples cut from the trees and transfers made to prune and oatmeal agars. Information from other parts of the United States where boxelder is cut in considerable quantity for commercial use indicates that this stain is very common and that it is a peculiar characteristic of this tree.

A preliminary note on the red stain of boxelder was published in March, 1922, by the writer in an article dealing with the economic aspects of certain stains commonly found in wood.[5]

The following taken from the article by Eidam(4) is of historical interest in connection with the red stain in boxelder; he says:

> Greek mythology speaks familiarly of the dryads, those nymphs who live in trees and are even said to suffer death with their felling. The ancient Greeks had been supported not a little in their poetic faith through the discovery of the blood red wood. We present-day skeptics take our microscope and prosaically attempt to probe the matter to the bottom.

Hedgcock [6] has recorded the occurrence of a pink stain caused by *Fusarium roseum* (group) upon various species of pine lumber, but no record is noted of its occurrence within the living hosts.

HOSTS

Boxelder, so commonly used as a shade tree, is the principal host of the organism producing red stain in the heartwood and to a less extent in the sapwood of the living tree. In this species of wood the stain has often been traced throughout the heartwood in freshly felled trees from roots two inches or less in diameter through the trunk into the main limbs and out into the branches which measured from one to three inches in diameter. Similar but paler discolorations have been observed in the wood of yellow poplar (*Liriodendron tulipifera* Linn.), gumbo limbo (*Bursera simaruba* (Linn.) Sargt.), aspen (*Populus tremuloides* Michx.) and in white pine (*Pinus* sp.).

Reports have been received of a red stain appearing near the juncture of sapwood and heartwood in white oak, but samples of this material

[5] HUBERT, Ernest E. SOME WOOD STAINS AND THEIR CAUSES. *In* Hardwood Rec., v. 52, no. 11, p. 17–19, 4 fig. 1922.

[6] HEDGCOCK, George Grant. STUDIES UPON SOME CHROMOGENIC FUNGI WHICH DISCOLOR WOOD. *In* Mo. Bot. Gard. 17th Ann. Rpt., p. 59–114, pl. 3–12. 1906.

have not been examined. Eidam states that a similar "blood red" discoloration was noted in a piece of beech wood, and records the finding by Stein of a "beautiful violet stain" in the wood of lilac (*Syringa vulgaris*). A bright violet-red color has been observed by the writer in a piece of lilac wood in the wood collection of the Forest Products Laboratory.

CAUSE OF THE DISCOLORATION

The discoloration in the wood of boxelder is due to a soluble pigment secreted by the fungus which stains the wood tissues and cell contents and by the presence in the wood of colored hyphae. The older hyphae within the wood tissues contain the coloring matter, as do also the hyphae in most of the cultures. An experiment was conducted to determine whether the coloring matter was to be found in solution outside the hyphal threads. Two tubes of malt agar on which the organism had been growing for a period of eight days were emptied of their contents upon a paper filter. Warm distilled water was poured over the agar and the collected filtrate showed a distinct reddish color. The formation of brighter colors is apparently favored by an acid medium, probably by the degree of acidity, since the areas of the heartwood showing the bright red colors react quite strongly acid to litmus, while the yellowish to brownish areas accompanying these react but slightly.

That the coloring matter diffuses out from the fungus and is not confined to the lumen of the hyphal cells is evidenced by the observations that hyphae are not always found in the red colored tissues. Apparently the colored liquid diffuses considerably beyond the hyphae which produce it. The color fades somewhat when a red-stained boxelder board is exposed for a year to sunlight.

DESCRIPTION OF STAIN

The red stain in boxelder varies considerably both in shades of color and in uniformity of distribution throughout the tree. The color ranges on moist wood from a light coral red to hellebore red or carmine.[7] On dry wood the hues are less intense and range from light coral pink to jasper red.

Very often the stain in the heartwood does not show a uniform coloring, but is broken by irregular blotches of various sizes and of a deeper hue (pl. 3). These blotches indicate individual infections due to sapsucker injury. Very frequently the heartrots caused by *Collybia velutipes* Curtis, *Pleurotus ulmarius* Bull., *Fomes applanatus* Fr. or other polypores are found in the heartwood (pl. 3). In such cases the red stain is found bordering the decayed areas and frequently the decayed area contains the red stain which had previously surrounded it but had become invaded by the advancing rot organisms. No particular signs of antagonism to one another is exhibited in wood containing the red stain fungus and a heartrot organism. When the red stain is present in the same areas with *F. applanatus* the latter fungus produces narrow black zone lines along the outer boundaries of the decayed areas (pl. 3), but these lines are not consistently formed, so there is no indication that they are due to a reaction between the two fungi. Similar lines are formed by *Fomes applanatus* in the absence of other fungi.

[7] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912.

Quite often the sapwood shows scattered, irregular patches of red stain which end abruptly at certain annual rings. These isolated stained areas are interpreted as individual infections originated through the wounding of the cambium by sapsuckers. The brighter tints of red are more commonly found in these areas.

The absence of hyphae in many instances in the outer borders of the discolored area leads to the belief that the coloring matter spreads through the wood ahead of the hyphae.

PATHOLOGICAL ANATOMY

Eidam, referring to the red stain in boxelder, states that if mounts are made of transverse and longitudinal sections taken from the red areas of the wood then the parenchyma cells are seen to be frequently penetrated by a fungous thread which is colorless, the walls of the tracheids are so corroded that they easily fall to pieces, and particularly in the large pitted tracheids of *Acer negundo* (the fungus threads) weave matted cushions of large anastomosed hyphae filling the cells completely.

A preliminary study of the material so far collected on boxelder indicates that the fungus is to be classed as a stain organism rather than as a wood destroyer. Microscopical examination of radial sections of the red colored wood taken from infected branches reveals the fact that the outer regions of the colored areas rarely contain hyphae. Occasionally, in the central area of the branch, where the fungus has been present for some time, hyphae were found in the vessels and in the pith cells. Penetrations of the pith cell walls were noted. In the pith the hyphae are irregular in size, rarely branched, and of a jasper pink to ochraceous salmon color.

No evidence of corrosion of cell walls such as observed by Eidam was noted in the material so far studied. Whenever corrosion was observed it was invariably attributed to the decay-producing organisms accompanying the red-stain fungus. A Myxomycete, which apparently feeds upon the hyphae of the red-stain fungus, is often found associated with the red stain. The question arises whether this Myxomycete may not be responsible for the scarcity of the hyphae of the red-stain fungus in the wood.

GEOGRAPHICAL DISTRIBUTION

The geographical distribution of this disease may be assumed to coincide with the range of the boxelder. The disease is widespread in this country throughout the States of Wisconsin, Minnesota, Michigan, and South Dakota. Few reports of its occurrence have been received from regions outside of the Middle Western and Southern States. It is commonly met with in the raw material of the slack cooperage industry. In Tennessee, where the writer visited a large cooperage mill, the boxelder bolts could be picked out of a carload of mixed stock by means of the vivid red color in the heartwood and sapwood. From published data a similar stain in boxelder appears to occur in widely separated countries in Europe.[8]

ECONOMIC IMPORTANCE

Since the red stain in the wood infected by this Fusarium constitutes a blemish,[9] the grade of such stained material is considerably lowered

[8] EIDAM E. OP. CIT.

[9] According to standard grading rules a blemish of this type consists of a stain either superficial or deep in the wood which is not sufficiently objectionable to be classed as a defect.

and the loss suffered is in proportion to the reduced price. For uses where bright stain-free stock is required, the red-stained wood is rejected. However, the stained stock is used for many purposes where the discoloration meets with little or no objection, or where it is covered or painted. The fact that this fungus is often associated with decay-producing organisms in the heartwood of boxelder should cause some hesitation in using stained stock for purposes requiring sound material.

THE CAUSAL ORGANISM

TAXONOMY

The causal organism has been isolated repeatedly in pure cultures by using fragments of the red-stained wood. Pieces of wood taken from the stained areas, the surfaces thoroughly sterilized by washing in mercuric chloride, 1–1,000, and then washed in distilled water, when placed in sterilized moist chambers invariably developed a white to pinkish mycelium in the red-stained areas adjoining the unstained wood and to a less extent in the remaining red-colored areas. Spores collected from this mycelium proved to be typical of the genus Fusarium. The various types of spores obtained on the malt, prune, and oatmeal agars by transfers from the primary cultures, gave additional proof of its generic identity. Cultures on malt agar and on oatmeal agar were sent to Dr. C. D. Sherbakoff, Experiment Station, University of Tennessee, who kindly determined the species and submitted the following description of the causal organism:

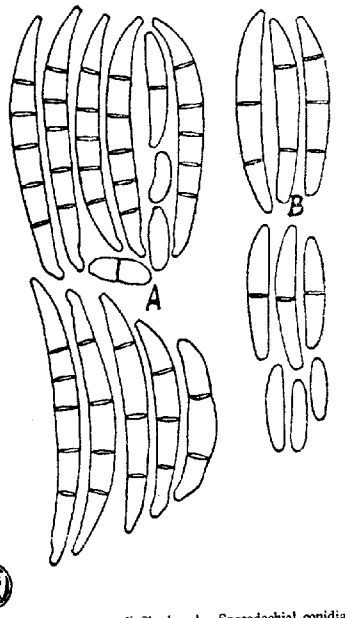


FIG. 1.—*Fusarium negundi* Sherb. A.—Sporodochial conidia from 30-day-old culture on oat agar plus 3 per cent glucose-maltose, in Petri-dish. B.—Free conidia from 3-day-old culture on corn-meal agar, in Petri-dish. Magnified 1,000 diameters. Drawing by Dr. C. D. Sherbakoff.

Fusarium negundi Sherbakoff (new species).

Sporodochial conidia 0 to 5-septate; 0 to 2-septate few, 3 to 5-septate common, 5-septate most numerous and measure 4.25 x 38.5 (4–6 x 34–42) μ; the spores are gradually attenuate toward the apex, pedicellate, somewhat more distinctly curved toward apex. Conidia borne singly on mycelial branches, few, 0–3-septate, ventrally nearly straight, apedicellate, apically attenuate. Aerial mycelium on most media in test tubes and in plates rapidly growing, even, fine, from white to carmine; substratum, in plates on agars with glucose, of carmine color. Large plectenchymic bodies (pseudo-sclerotia) common on oat agar. The sporodochial conidia much

resemble *F. incarnatum* (Rob.) Sacc., as per Wollenweber's figures in the supplement to his "*Fusaria* aut. *delineata*," but include none with more than 5 septa.

Habitat.—In red discolored wood of box elder, *Acer negundo* Linn., Madison, Wisconsin, United States of America.

Sherbakoff states that, "The general appearance of the fungus on hard oat agar in a test tube is shown in Plate 1. Free spore production on mycelium is very sparse and the conidia are of the type shown in Figure 1, B. Sporodochia in the media used are rarely produced, in fact only in one culture (a Petri-dish culture on hard potato agar plus 3 per cent dextrose-maltose) sporodochia appeared, and then in a comparatively large number, mostly one-fourth to 1 mm. in diameter, free, i. e., without a pseudoparenchymic base, with conidia of light-salmon color. When the culture was 8 days old the septation and size of conidia from the sporodochia were as follows: 0–septate very few; 1–septate, 1 per cent; 2–septate, not observed; 3–septate, 30 per cent, 3.5 x 31.5 (3.1–3.9 x 29–37) μ; 4–septate, 32 per cent, 3.85 x 35.7 (3.7–4.2 x 31.5–39) μ; and 5–septate, 37 per cent, 4.1 x 37.5 (3.8–4.2 x 35–40) μ. Another examination of conidia from the same sporodochia, when the culture was 30 days old, gave the following results: 0–septate, 2 per cent; 1–septate, 7 per cent; 2–septate, 2 per cent; 3–septate, 8 per cent; 4–septate, 9 per cent; and 5–septate, 72 per cent; the latter measuring 4.4 x 39.2 (3.9–6.1 x 34–42) μ. The conidia are shown in Figure 1, A."

MORPHOLOGY

The conidia (fig. 2, G.) are typical sickle-shaped spores with the characteristics as given by Sherbakoff. Macroconidia, microconidia, and chlamydospores are formed, both in artificial cultures and upon the exposed surfaces of the host, although up to the present time macroconidia have been found less frequently upon the host than the other forms.

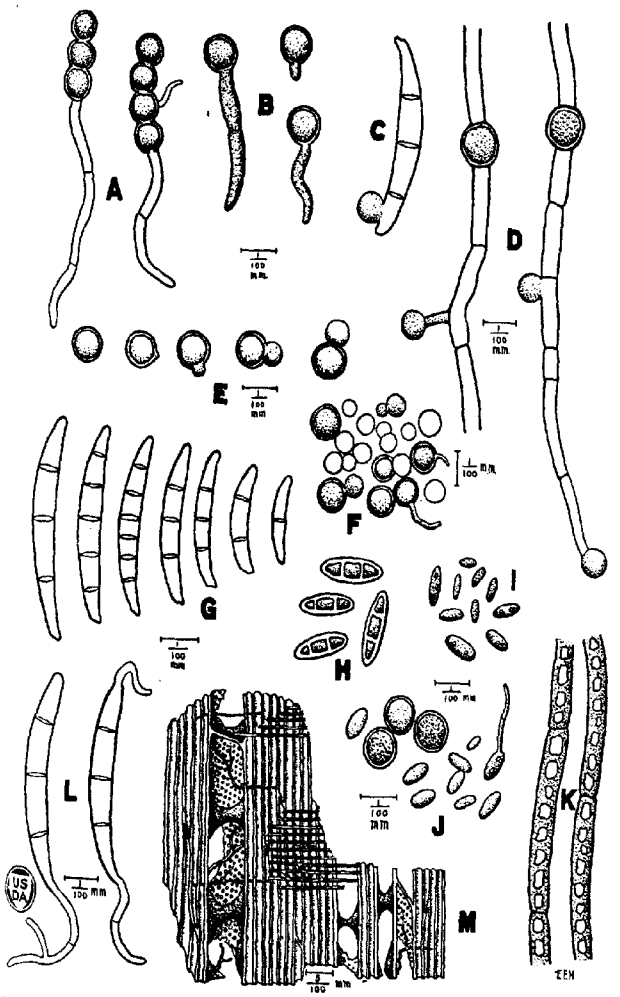
A six-day-old culture on malt agar, No. 91, when examined, showed large septate hyphae, constricted at the septa, with contents varying in color from yellowish to bright red and containing many large vacuoles (fig. 2, K.). Anastomosing of hyphae appears to be common in this species and reference to this character is made by Eidam.

The fungus develops readily from pieces of infected wood placed in moist chambers, and in most cases no great difficulty was experienced in securing pure cultures on various agars by using fragments of the discolored wood as inocula. On several occasions, however, the fungus has failed to develop from such fragments and this may be explained by the fact that microscopical examination of some of the stained wood discloses no hyphae within the tissues.

In eight-day-old Petri-dish cultures using malt agar the aerial growth covered the entire surface. From the under side the central area of the growth in Petri-dishes is of a pomegranate purple color and the outer, more recent, growth area an olive lake color.[10] The growth is more rapid and the discoloration of the substratum is more intense on prune and oatmeal agars than on malt agar.

Both terminal and intercalary chlamydospores are formed in cultures (fig. 2, D.). These spores may be single but more often are in chains

[10] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912.



IG. 2.—A. Chlamydospores in chains, showing germination. B. Chlamydospores, solitary, showing germination. C. Chlamydospore formation from one of the cells of a conidium. D. Various types of chlamydospore formation from partly dried cultures. E. Stages in the budding process of the chlamydospores. F. Chlamydospores taken from débris within a hollow knot, showing variation in size. G. Conidia from culture on oatmeal agar, showing variation in size and septation. H. Purplish colored spores from a dark crust-like layer found in a hollow knot of an infected boxelder. I. Microconidia from hollow knot. J. Chlamydospores with granular contents taken from a sclerotia-like structure (pseudosclerotia) found in culture No. 45, a few microconidia from the same culture. K. Hyphae with red-colored contents and large vacuoles from 60-day old cultures on oatmeal agar. L. Conidia germinating. M. Radial section through red, stained wood, showing hyphae in the tissues and penetration of cell wall.

(fig. 2, A and B.). Under certain conditions chlamydospores are formed from the cells of the macroconidia (fig. 2, C.).

Cover glass cultures, made by using transfers from culture No. 45, when examined under the microscope, showed chlamydospores of the fungus budding and finally germinating (fig. 2, E.). Chlamydospores taken from a reddish crustlike mass inside a hollow knot, when observed in hanging drop cultures, showed a similar budding process, resulting in the formation of large numbers of these spores (fig. 2, F.). Hyaline, one-celled and two-celled microconidia were also present and their germination noted (fig. 2, I.). The older mycelium produced in all these cases showed yellowish to reddish cell contents.

The fungus appears to develop rapidly under very moist conditions. This rapid growth was observed in the artificial inoculation of blocks of fresh sapwood placed in humidity chambers.

Cultural tests using pieces of red-stained boxelder kept in the air-dry condition of a room for a period of one and a half years show that the fungus is capable of reviving at the end of this period. Cover glass cultures made by placing microtome sections of the infected wood on a thin layer of agar under a cover glass showed that the new hyphae at the time of revival may originate from old hyphae or from chlamydospores formed in the tissues. Eidam states:

> In culture in the moist chamber the mycelium grows out from the wood and the brown hyphae put forth young colorless filamentous branches which phosphoresce very beautifully and distinctly so that thereby the whole outline of the piece of wood showed distinctly.

PATHOGENICITY

Numerous isolations of *Fusarium negundi* Sherb. in pure culture obtained from fragments of the stained wood prove the constant association of this fungus with this particular disease, which is characterized by a reddish discoloration. Comparisons of the hyphae and spores produced in pure cultures with those found in and upon the red-stained wood furnish additional evidence. Conidia (fig. 2, I.) and chlamydospores (fig. 2, F.) resembling closely those produced in pure culture were found on infected trees in the débris scraped from hollow knots and from cavities in the bark produced by species of sapsucker. Poured plate dilution cultures made from this spore mass developed colonies of a Fusarium which colored the agar a bright red and which were identified as *Fusarium negundi* Sherb. Information gathered in connection with sapsucker injury, leads to the opinion that the fungus is either weakly parasitic or develops on the injured tissues and produces discoloration of the surrounding sapwood tissues by diffusion of the colored matter which is in solution. By far the greater number of infections so far found in the sapwood of the living tree have their origin in the wounds produced by sapsuckers. (Pl. 2.)

The red stain was produced artificially in the laboratory on boxelder wood by inoculation with the fungus from pure cultures obtained from the red-stained wood. Difficulty was experienced in attaining positive results when heartwood of boxelder was used after sterilization by autoclaving for a period of 45 minutes at 15 pounds pressure. Better results were gained by using fresh sapwood blocks, surface sterilized by washing in mercuric chlorid and distilled water. Table I gives the results of these experiments. The fungus reisolated from a stained spot on one of the blocks was found to be identical with *Fusarium negundi*.

LIFE HISTORY

Not a great deal has been learned of the life history of this fungus. The presence of chlamydospores and conidia in hollow knots, in holes produced by sapsuckers, on the surface of broken branches showing red stain, and on dead wood exposed by wounding, indicates that these spores are produced upon the surface of the host wherever wounding and other factors have afforded suitable conditions. Undoubtedly many of the spores are wind or water borne, but judging from the activities of sapsuckers in connection with this host it is reasonable to suppose that these birds play an important part in disseminating the spores. A glance at Plate 3 will show a number of small red, stained areas in the sapwood between the bark and the continuous red area (dark band) surrounding the decayed heartwood. These areas are seen to be directly associated with "bird peck," a type of injury caused by the sapsucker in search of food. The evidence in Plate 3 shows that the same cavity is used at intervals to tap the cambial layer; in this case three annual rings intervene between two red areas which are directly in line with the hole drilled in the bark by the bird. The most recent injury, apparently produced in the spring of 1922, was not healed at the time the tree was cut in November of the same year. If these deductions are correct, then it is quite possible for the bird to transmit the fungus from one portion of the tree to another or from tree to tree.

The years of greater activity of this bird for a particular area on the tree can be measured by the larger number of bird-peck stain spots occurring along the same annual ring. The smaller spots represent the stained areas above or below the original injury and nidus of infection. The three blocks in Plate 3 show the "bird pecks" in longitudinal section of the wood.

In pure cultures the spores of *Fusarium negundi* Sherb. are produced within a period of three days. Under natural conditions sporulation could easily take place within the hole drilled in the bark by the sapsucker before the callus developed sufficiently to isolate the fungus within the sapwood. The next visit of the bird to the spot would result in a contamination of its bill with these spores.

Wounds caused by wind breakage, by pruning, by fire and by sapsucker attack, appear to be the most common infection courts for the entrance of this fungus. The part which insects may play in the life history of this stain organism has not been investigated.

The organism in the form of hyphae overwinters within the host tissue, renewing its activity upon the return of favorable temperature and moisture conditions.

CONTROL MEASURES

Sanitary measures are probably the only practicable means in controlling this disease on shade trees, providing the fungus is found to cause sufficient damage. Proper care of the trees in respect to the various injuries it suffers will aid greatly in reducing the chances of infection, not only of this disease but of the more serious heartrot and parasitic types. Wounds of all kinds should be given particular attention. Detailed information regarding the proper methods of caring for wounds on shade trees may be found in United States Department of Agriculture Bulletin No. 1178.[11]

[11] COLLINS, J. FRANKLIN. TREE SURGERY. U. S. Dept. Agr., Farmers' Bul. 1178, 32 p., 24 fig. 1922.

TABLE I.—*Results of infection experiments on wood of boxelder with pure cultures of the fungus, Fusarium negundi*

Experiment No.	Date.	Source of inoculum.	Medium and dimensions (inches).	Method of sterilization.	Number of tubes.	Results.	Date of results.
1	Apr. 7, 1921	Culture No. 91.	Blocks of heartwood, 1×1×5.	Autoclaved at 15 lbs. for 45 min.	3	Slight red stain in wood surrounding inoculum. Penetration of stain, slight.	May 9, 1921.
2	Apr. 7, 1921	Culture No. 45.	Blocks of heartwood, 1×1×5.	Autoclaved at 15 lbs. for 45 min.	3	Considerable surface staining of wood where hyphae developed. Penetration of stain, slight.	May 9, 1921.
3	Apr. 8, 1921	Culture No. 45.	Blocks of heartwood, 1×1×5.	Autoclaved at 15 lbs. for 45 min.	2	No staining. Hyphal growth scanty. Myxomycete strands developed from inoculum. Sclerotia-like growths on wood.	Apr. 26, 1921.
4	Apr. 7, 1921	None. Control.	Block of heartwood.	Autoclaved at 15 lbs. for 45 min.	1	No growth. No staining.	May 9, 1921.
5	Dec. 1, 1922	Culture No. 182.	Blocks of fresh sapwood, 1⅛ ×1⅛×2.	Surfaces washed with $HgCl_2$ and with distilled water.	3	Considerable surface staining in vicinity of inoculum. Penetration of stain into wood for a distance of ¼ inch.[a]	Dec. 14, 1922.
6	Dec. 1, 1922	Culture No. 182.	Blocks of fresh sapwood, 1¼ ×1¼×2.	Surfaces washed with $HgCl_2$ and with distilled water.	2	Considerable staining of surface and slightly below. Surface of block gives acid reaction.	Dec. 14, 1922.
7	Dec. 1, 1922	None. Control.	Block of fresh sapwood, 1¼ ×1¼×4.	Surfaces washed with $HgCl_2$ and with distilled water.	1	No staining. No hyphae of Fusarium developed.	Dec. 14, 1922.

[a] On Dec. 28 a Myxomycete developed and fruited on the surface of one of the blocks.

If it is found desirable to attempt the control of the disease on boxelder trees in the forest and wood lot intensive methods of control will be impracticable. Such sanitary measures as the burning of affected slash and rapid handling of the logs are steps which can be taken to reduce the number of inoculum sources. Rapid removal of the logs to the mill may reduce the production and dispersal of spores and rapid seasoning may check the development of the fungus in the wood.

SUMMARY

A disease of the boxelder characterized by a bright red stain in the wood has been under observation by the writer since 1920. The stain is very frequently met with and, therefore, popularly believed to be a fairly reliable character for the identification of this wood.

The cause of the discoloration ranging from light coral red to hellebore red or carmine in the heartwood and to a less extent in the sapwood is due to the presence in the wood of a soluble red pigment produced by the colored hyphae of a fungus, *Fusarium negundi* Sherb.

The fungus appears to be weakly parasitic since it is found developing in the sapwood following entrance through wounds principally caused by sap-suckers. The latter appear to be agents in the dissemination of the spores from different parts of a tree or from tree to tree. No evidence of penetration through living tissue in the absence of wounds has been noted.

For uses where bright, stain-free stock is required the red-stained wood is rejected. Presence of the stain may degrade the stock and reduce the price per thousand board feet. The association of the red-stain organism with fungi-producing wood rot in the same tree necessitates caution in the use of affected material.

The geographical distribution of the red-stain disease is assumed to coincide with the range of the boxelder. It has been found in many places in the United States, and what appears to be the same disease has been reported in a few places in Europe.

As means of preventing the discoloration of the wood and as a preventive measure in case the organism develops greater parasitic tendencies, sanitary measures directed to the proper care of wounds are suggested for shade trees; and for forest trees the burning of affected slash and the rapid handling of infected logs are believed to be of value.

PLATE 1.

Fusarium negundi Sherb. on oat agar, 56 days old. Hand painted by W. R. Fisher, of Cornell University. Natural size. Colored photograph furnished by Dr. C. D. Sherbakoff.

The Red Stain in the Wood of Boxelder

PLATE I

Journal of Agricultural Research

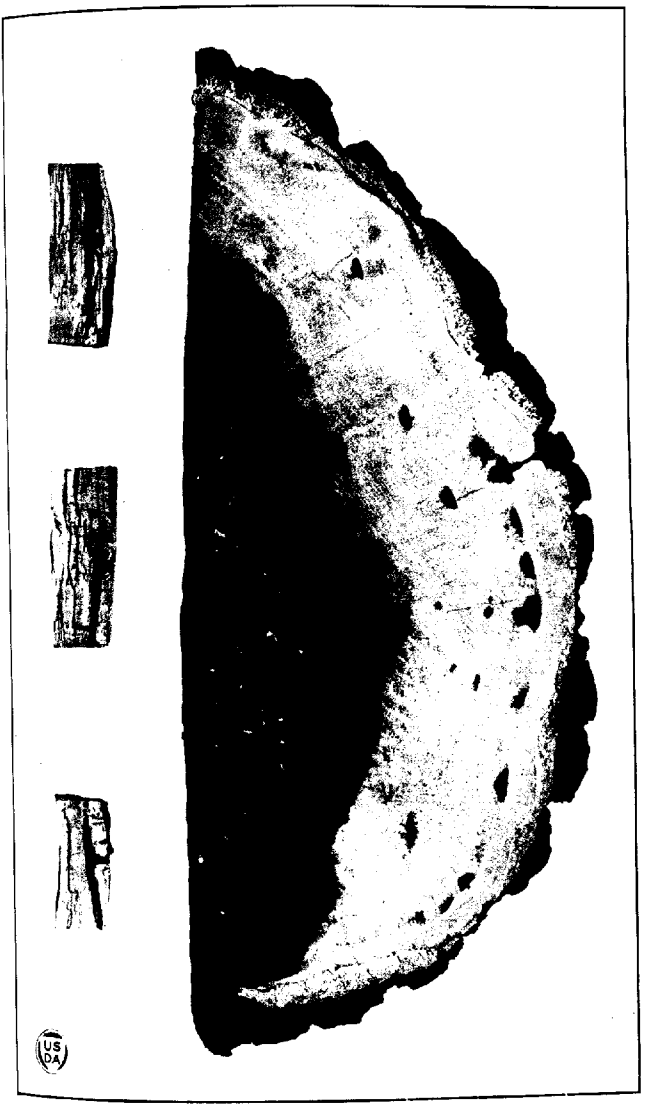
Washington, D. C.

PLATE 2.

Transverse section through the trunk of a boxelder showing the heartwood discolored by the red stain caused by *Fusarium negundi* Sherb.

PLATE 3.

Transverse section of boxelder cut down in November, 1922, showing the heartrot of *Fomes applanatus* in the central heartwood, surrounding this is a dark band of red stain with five projecting areas all halting abruptly on the same annual ring. The sapwood shows scattered individual infections by the red stain fungus which entered through the injuries produced by sapsuckers. At the division line between the two annual rings last formed are found eight of these infections. One of these injuries had not been healed and the direct relation is shown between the red stain area and the cavity in the bark and cambium produced by the bird. Three of the "bird pecks" are shown in longitudinal section of the wood.



STEM AND ROOTROT OF PEAS IN THE UNITED STATES CAUSED BY SPECIES OF FUSARIUM[1]

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INTRODUCTION

It is a well known fact learned through the costly experience of pea growers in the older portions of the United States that in many localities when peas are grown repeatedly on the same ground the time comes sooner or later when they thrive less vigorously, and finally fail completely. After such a failure, the ground must be devoted to other crops for several years before peas can be grown again with any degree of success, and often the ground appears to be permanently ruined for pea growing. Experience similar to this has long been known in Europe and Asia wherever peas are grown; but it appears to have been felt more keenly in America where the development of the canning industry has stimulated the intensive culture of peas in small areas close to canning establishments.

This failure of peas is always found upon examination of the plants to be due to a decay of the base of the stem and of the roots of the plants. The cause of this decay has been sought by a number of investigators in Europe and America, and a considerable list of parasitic fungi have been accused on the basis of evidence of varying value. These several investigations, conducted in limited areas and arriving at diverse results, have not furnished pathologists criteria whereby they may either determine which of the several diseases are present in any new locality, or initiate control measures on the basis of a knowledge of the life history of the parasite to be combated. Most important of all, no evidence has been provided which can indicate whether these diseases can be kept out of new pea-growing regions that are being developed. This situation led in the summer of 1918 to the assignment of the writer to the task of determining the parasites causing decay of roots and basal portions of the stems of pea plants in the pea-growing regions of the United States where trouble has been experienced. As a result of this investigation, which is now approaching completion, it has been found that four parasitic fungi are the chief factors in producing decay of the underground portions of the pea plant in all the localities examined. These fungi are a species of Fusarium previously found but not named by G. R. Bisby in Minnesota, an undescribed species of Aphanomyces, *Pythium debaryanum*, and *Corticium vagum*. Although these four fungi usually occur together wherever rootrot of peas is serious, and although they cause diseases that can not always be distinguished from each other with certainty by visible symptoms, yet these four fungi are factors of such distinct character in their contribution to crop failures that they will be discussed separately. This paper deals with the disease caused

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by the important parasite Fusarium, here described as a new variety of *Fusarium martii* App. and Wr., and contains notes upon other species of Fusarium which have been isolated frequently from diseased pea plants, or which have been mentioned in literature as parasites. The diseases caused by the other parasites mentioned above will be treated in subsequent papers.

THE DISEASE

DESCRIPTION

The disease caused by Fusarium, unfortunately, does not exhibit any symptoms upon the aerial portion of the plant that are different from those produced by several other diseases. A considerable decay of the cortex of the stem occurring late in the development of the plant may not cause any apparent unfavorable effects upon growth. If many roots are destroyed, growth may be retarded, and an invasion of the vascular bundles of the stem may cause a wilt of the entire plant. Since the invasion of a plant by one of the other parasites may cause all of these degrees of injury, and the other diseases may produce some of them, the distinguishing characteristics of the disease must be sought in the region which the fungus has penetrated.

The most susceptible portion of the plant is the base of the stem above the point of attachment of the cotyledons. The largest amount of damage is done when the fungus enters at this point and causes such disintegration of the tissues that the taproot is separated from the stem. At higher soil temperatures the vascular bundles turn a characteristic reddish brown, and the plant wilts; at lower temperatures the connection between root and shoot may be completely rotted off, forcing the plant to depend entirely upon roots which are developed above the point of injury. The lesion which the fungus produces at its point of entry is easily distinguished, at least in its early stages, by its color and shape. In color it is reddish brown or chocolate, in form it is elongate, often wedge-shaped with the base of the wedge at the point of attachment of the cotyledons and the apex pointing upward. Lesions are not sunken until they are extensive. If the lesion reaches the vascular tissue this takes on a bright orange red or brown color that may extend above the external lesion as far as the first node. The lesion caused by Fusarium is distinguished from that caused by the phycomycetous fungi by its darker color, and from that caused by Rhizoctonia by its shape, and the absence of a sunken eroded surface.

The disease is found as a decay of the taproot or of any of the smaller roots. Dark lesions occur along the roots and the ends of roots are killed, but this form of the disease can not be distinguished readily from that caused by Rhizoctonia.

ECONOMIC IMPORTANCE

It is not difficult to discuss the economic importance of the entire group of diseases causing decay of the roots and base of the stems of the pea plant. The present recognition by pea growers of the necessity for the rotation of crops has been brought about by very costly experience to the factory owner and grower alike. Even now the number of fields damaged by disease is considerable, even in districts where most intelligent care is taken in the selection of suitable fields for the crop.

Since the disease caused by Fusarium is only one of four diseases which are usually operating jointly to bring about the economic consequences indicated, and since these diseases do not have distinct characters which enable one to differentiate them with certainty, a statement of the relative importance of any of them is at present largely a statement of personal opinion which must be held subject to revision. Taking into consideration all the territory that has been examined, the writer is inclined to believe that the diseases due to the two phycomycetous species cause by far the largest part of the loss; that the disease caused by Fusarium is second in importance, while that caused by *Corticium vagum* is of much less importance than either of the preceding. Local variations in this order of importance are brought about by environmental conditions which especially favor one or another of these diseases.

DISTRIBUTION OF THE DISEASE.

All of the root parasites of peas are nearly coextensive in their distribution. The disease caused by the species of Fusarium has been found in scattered localities near the Atlantic coast from Maine to Florida, in all the North Central States and Minnesota, and in Montana and Utah. Search has not been made in any of the Pacific Coast States. The only important pea-growing district that has been searched in vain thus far is in Idaho. The dissemination of the disease within the districts where it occurs varies greatly, depending apparently upon the two factors—the length of time during which peas have been grown in that district and the frequency with which they have been planted on the same land. There are few localities which have been examined in which peas have been grown more than 10 years intensively upon narrowly limited areas in which this disease has not become a more or less important factor which is reducing yields. The climatic conditions which determine, in large measure, the amount of damage that it may do are discussed later. For the present it is sufficient to say that the disease is distributed very thoroughly throughout the most of the pea-growing area of the United States.

PREVIOUS RECORDS OF PEA DISEASES CAUSED BY SPECIES OF FUSARIUM

Serious stem and rootrots of peas caused, or believed to be caused, by species of Fusarium have been noted several times in Europe and America, and have been studied at several points in Europe. The first of these to receive serious attention was the so-called St. John's disease of peas in Holland reported by Van Hall in 1903 (*8*).[2] The description of the disease is not given in sufficient detail to enable us to distinguish it from other diseases now known. It is said to remain in spots in fields for a long time. From dying plants Van Hall isolated a Fusarium which he regarded as very similar to *F. vasinfectum*, Atk. and which he designated variety *pisi* of that species without description. The pathogenicity of this fungus was tried in a single experiment upon plants grown in water culture, though Van Hall admits that the infection which he obtained in this manner is not an adequate proof of pathogenicity.

Later Schikorra (*11*) found what he believed to be Van Hall's St. John's disease. He mentions a yellow color of the center of the stem above

[2] Reference is made by number (italic) to "Literature cited," p. 475.

ground which gives good evidence that a Fusarium was present. A species of Fusarium was isolated, and a single inoculation of 20 seeds in one pot of sterile soil was made. All of the plants became infected in eight weeks. The larger part of Schikorra's paper is devoted to studies of the physiology of this organism, which he regards as identical with Van Hall's fungus. Fortunately we have a good description by Appel and Wollenweber (*1*) of the fungus with which Van Hall and Schikorra worked. They have included the organism in their new species, *F. falcatum*. Later Wollenweber (*14*) states, apparently upon the evidence of his own experimental work which is not described, that "more than one species, differing both in size of conidia and color of conidial mass may cause the St. John's disease of the garden pea." This disease has since been reported in Europe by Guéguen (*7*) in France, and by Mortensen et. al. (*10*) in Denmark.

More recently Turesson (*13*) in Sweden has reported a disease of peas caused by *Fusarium viticola* upon a basis of evidence that appears to be adequate. The disease occurred at the plant-breeding station at Svalöv after a period of unfavorable weather. The trouble began at the neck of the root, often as a dark red discoloration, and spread up and down until in many cases the plant wilted. *Fusarium viticola* was isolated, and plants were inoculated in several ways with varying success. Soil inoculation always gave positive results. Varieties of peas seemed to show considerable variation in susceptibility to infection.

In American literature there are several notes referring to species of *Fusarium* associated with diseased peas (*5*, p. 202), though proof of the pathogenicity of the fungus is lacking in all but a single case. In 1911 Gifford (*6*, p. 151) makes the unsupported statement that he has found a disease of peas caused by *Fusarium*. Lewis (*9*) reports having isolated *F. orthoceras*, as determined by Wollenweber, from a diseased pea plant. In 1913 Wollenweber (*14*) describes *F. redolens* as a "vascular parasite, cause of wilt and foot disease of *Pisum sativum*," on the basis of his own work, which is not described. In the following year Wollenweber (*15*) describes *F. oxysporum* as occurring on *Pisum*, though he evidently does not intend to state that it is a parasite. Finally, Bisby (*2*) notes a disease of peas caused by a species of *Fusarium* in Minnesota, and later (*3*, pp. 19–20) he reports having found one species belonging to the section Martiella of that genus particularly pathogenic. The writer has received a culture of this fungus from Doctor Bisby, and finds it identical with the organism described in this paper.

THE FUNGUS

DESCRIPTION.

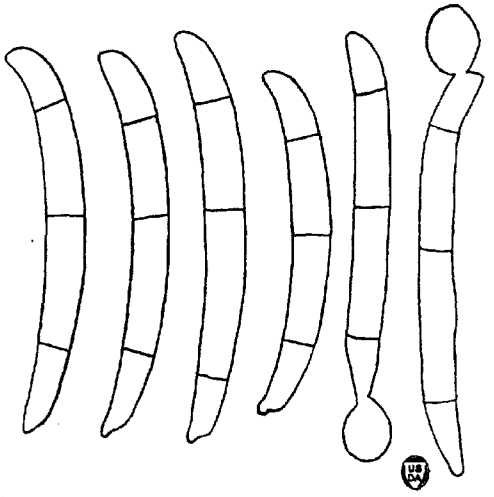
The following description of *Fusarium martii* App. and Wr. var. *pisi* (n. var.) is made from the fungus growing upon culture media, since it has never been observed to fruit on the plants that it infests.

Aerial mycelium short, white or grayish, sometimes absent when spores are abundant. Pseudopionnotes or sporodochia methyl prussian, zinc, or invisible green, sometimes avellaneous when young. Macroconidia mostly 3-septate, 27–40 x 5–4.5 microns, nearly uniform in diameter, typically more curved toward the apex; microconidia present, not abundant. Chlamydospores present in mycelium and in older spores;

mycelium intercalary or terminal, singly or in chains, 8–10 microns in ameter. Sclerotia absent or very rare on old rice cultures only.
Pathogenic in varying degree upon *Pisum sativum*.
Differs from *F. martii* App. and Wr. in having smaller spores, and om Sherbakoff's variety *minus* of that species in the smaller diameter its spores, comparative scarcity of sclerotia, and in the predominance green and blue color in conidial masses.

CULTURAL CHARACTERS

When the fungus is in a condition of "high culture," and it is not fficult to maintain it in this condition, its appearance upon the various dinary culture media does not differ greatly. The predominant green



IG. 1.—Spores of *Fusarium martii* var. *pisi*. The last two spores at the right have developed chlamydospores. X 1455.

blue color of the larger part of the spore mass is a conspicuous char-ter, at least in older cultures. Production of color varies a little with different strains. The substrate is not greatly discolored, though on tato agar rich in dextrose a vinaceous coloration is often produced. rice, it appears that at least two pigments are produced in varying portions. In the earlier development of the fungus a blue color is n at the lower edge of the advancing mycelium, while a vinaceous or is seen predominantly at the surface. These two colors are finally ngled through the rice in varying proportions. Sometimes the vina-ous color predominates, producing a brownish vinaceous color. If the e predominates, vinaceous drabs are produced. Tendency toward dominance of one or the other of these colors seems inherent in ains of the fungus from different sources.
The morphology of the spores is not much changed upon culture media, naining typical even upon rice (fig. 1). Characteristic measurements shown in Table I.

TABLE I.—*Characteristic measurements of spores of Fusarium martii (App. and Wr.) var. pisi (n. var.) from pseudopionnotes produced in cultures 20 days old*

Septation.	On oat agar.			On potato agar.			On potato agar with 2 per cent dextrose.		
	Maxima and minima.		Average.	Maxima and minima.		Average.	Maxima and minima.		Average.
	P. ct.	μ	μ	*P. ct.*	μ	μ	*P. ct.*	μ	μ
0	1	9 to 12×2 to 2.5	11×2.4	12	5 to 9×2 to 3	7×2.3	5	6 to 11×2 to 3	9×2.1
1	1	.....	.....	5	15 to 27×2 to 4	20.4×3.5	5	.....	.....
2	10	24 to 28×4	.....	29	29 to 33×4	30.6×4	0	.....	.....
3	85	27 to 37×4 to 4.5	31.7×4.3	54	29 to 38×4	32×4.2	93.5	27 to 37×4	33×4
4	3	33 to 42×5 to 4	37.2×4.2	.....	.....	.....	1	34 to 42×4	39×4

TAXONOMY

The foregoing description of the fungus causing the stem and rootrot of peas corroborates Bisby's opinion that it belongs in the section Martiella of the genus Fusarium, and that it is very closely related to *F. martii*. It is not identical with any of the varieties of that species described by Sherbakoff (*12*). An opinion must be rendered whether the differences found entitle the fungus to a specific rank or whether it should be placed among the varieties of *F. martii*, two of which have been distinguished by Sherbakoff on morphological grounds, and one by Burkholder (*4*) by a physiological character, pathogenicity toward varieties of *Phaseolus vulgaris*.[3] Since the fungus in question varies greatly in pathogenicity toward species of Pisum, it must be distinguished on morphological characters which are constant. It appears to the writer that the differences between this fungus and *Fusarium martii* are not greater than those between the species and varieties already described by Sherbakoff, and therefore the fungus is considered to be a variety.

HOST PLANTS

All of the limited number of species of the genus Pisum that have been available for study have been found susceptible to infection, at least at the base of the stem. No cases of strongly marked resistance at this point have been found. The following host list has been studied: *Pisum sativum* Linn. var. *arvense*, P. *sativum* Linn. var. *saccharatum*, P. *sativum* Linn. var. *umbellatum*, P. *elatius* Bieb., P. *jomardi* Schrank.

Lathyrus odoratus has been slightly infected, but so slightly that it does not seem likely that this fungus ever produces an important disease of this plant.

PHYSIOLOGY OF THE FUNGUS

This variety of *Fusarium martii* does not appear to possess any unusual characteristics in the germination of its spores or in any other function that merit special attention. However, in connection with a study of the relation of soil temperature to the development of the disease, it became a matter of interest to determine the optimum temperature for the growth of the fungus in pure culture. It was grown several times in petri dishes on potato agar with 2 per cent dextrose in a series of incubators maintained at temperatures that were approximately con-

[3] Burkholder (*4*) has suggested that since Dr. Westerdijk isolated a certain culture of *Fusarium martii* from peas there may be a *F. martii var. pisi* parasitic upon peas. Such a physiological variety has not been demonstrated or described.

tant. The following table gives the average diameter of the colonies fter seven days in a typical series.

ABLE II.—*Diameter of colonies of F. martii var. pisi grown on potato-dextrose agar seven days at the temperatures designated*

emperature (°C.)	10 to 11	12 to 14	15 to 16	19 to 20	24 to 25	30 to 31	33 to 34	36 to 37
iameter (mm.)	14	18	24	44	68	74	57	16

The optimum temperature for mycelial growth is here shown to be etween 20° and 34° C. Minute growth has been observed as low as -6° C. Spores are produced at all temperatures at which growth occurs.

ELATION OF ENVIRONMENTAL FACTORS TO THE DEVELOPMENT OF THE DISEASE

SOIL TEMPERATURE

The first preliminary inoculation experiments which were made gave sults which indicated that soil temperature modified greatly the pidity with which the disease developed. In order to determine, in a reliminary way, the range of temperature through which infection kes place, planting was made as follows in soil held at controlled tem- eratures in the Wisconsin soil temperature tank. One 5-inch can at ch temperature filled with soil from a field in which peas had never en grown, and which in previous trials had given plants free from isease, was planted with 7 Alaska peas. Two cans were filled with a ixture of 5 parts of this soil with 1 part of the same soil previously oculated with spores of the Fusarium, and in which diseased plants d been produced. The amount of moisture in the soil was held approxi- ately constant through the experiment.

Visible symptoms of disease developed first in the plants grown in oculated soil at 27° C. in 20 days after planting, when 4 plants wilted wn, and were found upon removal to be completely rotted off at the tyledons, and to have vascular discoloration extending above the soil rface. From this time on plants continued to die with vascular fection at 27° and 30°, and finally after 35 days three plants wilted at ° C. The experiment was concluded at the end of 43 days. The mber of plants living and dead at each temperature was as follows:

BLE III.—*Number of pea plants living and dead 35 days after planting in inoculated and uninoculated soil at a series of soil temperatures*

Temperature.	In uninoculated soil.		In inoculated soil.	
	Living.	Dead.	Living.	Dead.
°C.				
0	7	0	3	10
7	7	0	2	11
4	6	0	8	5
1	7	0	14	0
8	6	0	12	0
5	6	0	14	0
2	7	0	13	0
	6	0	13	0

All remaining plants were washed from the soil and examined. The controls showed at higher temperatures a few unimportant lesions at the cotyledons, of a very different nature from that caused by the Fusarium. All remaining plants in the inoculated soil at and above 21° C. showed red-brown shrunken stems for a distance of from 1 cm. at 21° C. to 2 cm. at 27° C., where roots were also extensively blackened. At 18° C. all plants were nearly or quite girdled by superficial lesions, which did not penetrate to the vascular bundles. At 12° C. small brown lesions were found from which the fungus was isolated.

Since this experiment indicated clearly that soil temperature controlled the development of the disease, three subsequent series upon a larger scale were placed in the tanks for the purpose of obtaining more extended data. Two of these series were in soil supposed to be free from fungi which infect peas, but the results indicated that this was not invariably the case. Not only did Rhizoctonia occur, but other species of Fusarium which were shown to have almost no ability as parasites by themselves entered the exposed vascular system of plants damaged by the parasitic species of Fusarium, and caused a more rapid wilting than the parasite alone could bring about.

Setting aside these results which were clearly brought about by the accidental introduction of minor parasites, the results were in entire accord with the final series in which steam-sterilized soil was used.

At each of the temperatures in this series five pots were planted with 10 Alaska peas each. Three pots were inoculated by spraying the seed as planted with a suspension of spores of the same strain of this Fusarium that had been used in the previous series. The dates at which plants wilted are shown in the following table. These dates are necessarily somewhat irregular inasmuch as badly diseased plants will remain turgid for a long time during cloudy weather and succumb suddenly when sunshine falls upon them. It will be seen from this table that while wilting begins at 24° C. almost as soon as at the higher temperatures it soon diminishes. This is due to the fact that the diseased plants have begun to send out roots from above the point of injury, so that from this time forth they can obtain moisture for maintenance and slow growth through these roots at the surface of the soil, even though the stem is completely rotted off below them.

TABLE IV.—*Record of the dates at which pea seedlings inoculated with Fusarium martii var. pisi wilted at each of the soil temperatures maintained* [a]

Temperature.	January.							February.							
	20	22	23	25	27	28	31	3	4	5	8	9	11	Plants remaining.	Plants dead.
°C.															
33	....	....	3	....	....	....	....	6	1	....	2	7	2	3	2
30	....	6	5	5	2	1	2	3	2	....	....	....	1	0	2
27	1	1	6	3	1	2	4	3	4	1	1	....	1	1	2
24	....	....	3	2	2	....	....	4	1	....	1	....	....	11	1
21	....	....	....	....	....	....	....	....	....	....	....	....	....	24	....
18	....	....	....	....	....	....	....	....	....	....	....	....	....	24	....
15	....	....	....	....	....	....	....	....	....	....	....	....	....	24	....

[a] 30 Alaska peas were planted at each temperature on Jan. 6.

Table IV, showing the temperatures at which wilting took place, indicates very clearly the optimum temperature range for the development of the disease. It extends from 24° to 33° C., the upper limit of temperature at which the pea plant will thrive. But damage to the pea plant occurs below this optimum range. At the conclusion of this series all plants except those grown at 15° C. were washed from the soil and examined. The remaining plants at 24° C., which were dwarfed, showed decay of the cortex at the base of the stem, including usually a discoloration of the vascular system beneath the decayed area, but not extending above. Had they been allowed to continue to grow there is little likelihood that they would have survived to produce seed.

The plants grown at 18° C. soil temperature showed very slight superficial discoloration of the cortex. They had suffered very little injury. Those at 21° C. were for the most part discolored for a short distance above the attachment of the cotyledons all the way to the vascular system, and some had discolored vascular tissue beneath the decayed cortex. In order to determine if these plants had suffered retardation of growth up to this time, the tops and roots were weighed separately. The result is given in the following table:

TABLE V.—*Average dry weight in grams of tops and roots of pea plants grown from January 6 to February 12 at the soil temperatures indicated in steam-sterilized soil and in soil inoculated with Fusarium martii pisi*

Temperature.	Treatment.	Number of plants.	Average weight of tops.	Average weight of roots.
°C.			*Grams.*	*Grams.*
21	Control	16	.208	.045
21	Inoculated	24	.192	.048
18	Control	17	.198	.029
18	Inoculated	24	.200	.036

It is readily seen from this table that up to this time the apparent damage had not produced any material retardation in the development of the inoculated plants.

The plants from one of the pots of inoculated soil held at 15° C. were washed and no trace of injury was discovered. The remaining two pots of inoculated plants and two pots of controls were transferred to the tank maintained at 27° C. until the conclusion of the experiment two weeks later, when the plants were in full bloom. The inoculated plants were then notably shorter and less thrifty in appearance. The bases of the stems of the inoculated plants were brown and shrunken with discolored vascular strands in a few cases. The dry weight of the tops of 16 inoculated plants was 7.1 grams, while that of the same number of controls was 8.67 grams. The root systems were almost exactly equal in weight. Thus these inoculated pea plants had begun to suffer from a relatively brief period of temperature favorable for the development of disease, even though wilting had not occurred. In all inoculation experiments wilting of pea plants has rarely resulted when the fungus has gained access to the vascular system after the early stages in the development of the plant.

These experiments in which the effect of an extended range of soil temperatures upon the development of the disease has been determined

have not only reproduced the disease as it occurs in the field, but have shown other effects rarely observed in the field. Chief among these is the wilt of seedlings at high soil temperatures consequent upon either a complete rotting off of the base of the stem, or more usually an invasion of the vascular system of the subterranean portion of the stem by the parasite. This invasion, it may be noted, is not in a manner typical of vascular parasites, inasmuch as it follows a very extended decay of the outer tissues, is somewhat limited in the distance to which it progresses, and is often preceded rather than followed by discoloration. When a similar decay of cortical tissues is produced by other organisms it is not uncommon for any one of a number of species of Fusarium to advance as far and produce a wilt. The more important information contributed by the temperature studies is an explanation of the varying importance of the disease in regions where it occurs, and the aid which this knowledge gives in distinguishing the several pea diseases. The disease can not become important in most pea-growing sections of Montana, for instance, and in regions where the growing season is continuously cool because of the low soil temperatures. It can not be the cause of the decay and death of plants that often occur in early spring before warm days have arrived. This disease requires a higher temperature for its inception than that caused by any of the other parasites studied.

RELATION OF SOIL MOISTURE TO THE DEVELOPMENT OF THE DISEASE

In order to determine whether high water content of the soil increases or decreases the rate of development of this disease, one can of inoculated soil placed at each temperature in a series similar to that previously described in detail, was maintained at about 75 per cent of its water-holding capacity, while the other cans were maintained at 50 per cent of the water-holding capacity of the soil. The plants in the wet soil did not show any marked difference in behavior from those in the drier soil. Peas have been grown in saturated soil at an optimum temperature for the development of the disease. Here wilting seems to take place a little earlier than in drier soil, evidently because the damaged tissues become water soaked and destroyed by bacteria at a more rapid rate. While in the case of this disease, as in the case of others, wet soil promoted decay started by the parasite, it does not appear to affect in great measure the action of the parasite itself.

VARYING PATHOGENICITY OF CULTURES OF *FUSARIUM MARTII* VAR. *PISI*

During an extensive search for this Fusarium in pea-growing districts in 1920, an isolation from pea roots grown in the Bitter Root Valley, Montana, gave a culture which, when used for inoculation, gave very slight infection. Thereupon spores from all of the cultures of this fungus which had been collected were used to inoculate peas under controlled optimum conditions for infection in order to compare their pathogenicity. The culture from Montana produced but few slight lesions, a culture from Maryland caused a mere browning of the susceptible portion of the stem, and a third culture from Madison, Wis., was hardly more of a parasite; while nearly all of the plants inoculated with other strains were rotted off at the attachment of the seed. Since two of these non-

pathogenic strains were obtained from districts where rootrot is not severe, it seemed possible that there might be a relation between the pathogenicity of the strain of Fusarium present there and the severity of the disease. The following year the collection of cultures was enlarged, not by direct isolation of the fungus from diseased stems—a procedure which is difficult when materials are not fresh—but by placing diseased stems in sterile soil, maintaining optimum conditions for infection, and isolating the fungus from diseased seedlings which resulted. Two new cultures obtained in this way from the Bitter Root Valley were much more pathogenic than that of the previous year, though one of them was much less pathogenic than the strains from Wisconsin and Michigan that were used as standards of comparison. Repeated inoculations have established beyond doubt the fact of the slight pathogenicity of the cultures enumerated, and have indicated that there is a constant, though often slight, difference between the more pathogenic cultures.

If, then, this difference in parasitism, whatever its physiological significance, has not been produced by the method of isolation or by conditions of culture, but inheres in the fungus in the field, such a fact is of importance, inasmuch as upon the degree of parasitism depends the degree of injury that the disease may cause. The existence of parasitic and of nonparasitic varieties of fungi are well known; but instances of intermediate degrees of parasitism have not been extensively investigated. We can not, then, obtain clues from past experience which will incline us to expect to find these differing strains constant in parasitism in the soil as they appear to be in culture, or mingled together in the same field, or constant over considerable areas. Neither do we know whether the constant presence of a susceptible host increases the degree of parasitism of any portion of the potential parasite in the field. Unfortunately, the writer has not secured a sufficient number of cultures for comparison to obtain a clue to the answer to any of these implied questions. However, the finding of such variability in one species is not likely to be a unique experience; and thus the result of inoculation with a single or even a few local isolations, at least of a Fusarium, does not necessarily determine more than a local pathological significance of that fungus.

RELATION OF SOIL CONDITIONS TO THE PERSISTENCE OF THE FUNGUS

The experimental inoculations conducted under controlled environmental conditions in the greenhouse have presented convincing evidence of the pathogenicity of this Fusarium when newly infested soil is planted with peas. The relation of the fungus to the host plant when the fungus is abundant in the soil can be worked out with comparative ease; but the relation of the fungus to its natural environment in the field—that relation which determines not only the abundance but the persistence of the fungus in the soil—can not be determined in a brief space of time by laboratory methods. The absence of conidia by which the fungus can be distributed, and its apparent absence from seed, leave us to assume that it persists chiefly as mycelium, which can only be detected by the presence of a host plant in which it may produce lesions. The number or extent of lesions becomes, then, the only criterion whereby we can determine from month to month or from year to year in inoculated or infested soil the vegetative activity of the fungus.

In order to obtain field evidence of the persistence of the fungus in different types of soil and to provide suitable plots for the trial of varieties of peas for resistance, a number of plots of soil supposedly free from all root-infesting parasites of peas were inoculated with the fungus and planted with inoculated seed in much the same way as in the greenhouse trials already discussed. Inasmuch as certain of these plots could not be critically examined later for results while others were found infested with other parasites which made results valueless, only two plots at Madison are regarded as highly significant. Since no inoculations with Fusarium, with one possible exception, have given infection that affected yields, the progress of the disease has been determined by examination of plants removed from the soil from time to time for the reisolation of the fungus. Among the inoculated plots besides those at Madison, Wis., was one at Arlington Experiment Farm, Va., planted first in 1919 on well drained clay soil. The writer was unable to observe the peas grown on this soil in 1920, but in 1921 peas grew without a trace of infection from this fungus. A small inoculated plot at McMillan, Mich., planted first in 1920 on sandy soil low in humus, gave plants with only a few slight lesions on the bases of the stems, though in a field close by, on similar soil high in organic matter and long used for pea growing, there was much of the disease present. At Madison only two plots remained free from other diseases during three years, thus permitting observations of the effect of Fusarium alone. One of these plots was on a well drained gravelly loam low in humus; the other was on a reclaimed marsh where organic matter was abundant. On the loam 12 varieties were planted on April 25, 1919, in rows 20 feet long, the soil and seed in one-half of each row being inoculated with the same strain of the Fusarium that had been used in the greenhouse inoculations. Three of the early varieties showed a few dying plants toward the end of the growing season, but on the whole it was difficult to distinguish by appearance the inoculated from the noninoculated plants. Peas were returned to this ground the following year with even less infection. In 1921 peas on the inoculated soil showed no typical Fusarium disease and could not be distinguished from controls when roots were examined. Since the ground was not available for peas the fourth year, some of the soil was brought into the greenhouse during the following winter and planted with peas at an optimum temperature for the development of the disease. No infected plants were obtained. A similar plot of soil inoculated in 1920 gave plants free from disease in 1921.

That this type of soil was not unfavorable for the development of all root parasites is demonstrated by the fact that two short rows inoculated with soil brought direct from diseased fields gave plants showing visible symptoms of disease produced by Aphanomyces sp. and the amount of injury to plants increased in the two succeeding years, spreading to adjacent rows.

The other plot started in 1919, at Madison, was on muck soil and was not planted until June 30. As might be expected, because of the high temperatures prevailing at that time of year, all the plants were rotted off at the seed and managed to maintain a stunted existence only by means of rootlets sent out above the point of injury. This area was replanted in 1920 without further inoculation, and the disease appeared as destructively as in the preceding year. The second replanting in 1921 still gave disease in undiminished severity, equaling that in a newly inoculated plot, while the control plot remained free from disease.

It is unfortunate that other plots on different types of soil were invaded by more vigorous parasites and ruined for comparative study of Fusarium injury. The simplest interpretation of this single comparison is that the presence of much organic matter in the soil favored the persistence of Fusarium. This interpretation is favored by field observations which, when reviewed, show that the largest amount of damage from this fungus is, in as far as it can be distinguished, on soils high in organic matter. Whatever factors determining persistence may ultimately be distinguished, the fact is clearly established that the parasite is dependent upon soil conditions for its persistence, and that it is not always or perhaps even frequently able to become a limiting factor in pea culture. Even in the soil most favorable for its persistence that was found, its spread through the soil from year to year was small, amounting to only about 2 or 3 feet in two years.

RESISTANCE OF VARIETIES OF PEAS TO FUSARIUM

Inasmuch as it is a well known fact that Canada field peas appear to suffer less from root diseases than canning varieties, and that canning varieties differ among themselves, an attempt was made to determine if this difference in resistance is due to difference in resistance to this Fusarium. The varieties first compared were Alaska and Rice's No. 330, varieties which Dr. Wilber Brotherton, jr., had found to show great difference in vigor in a field infested with several root parasites. After several preliminary trials had failed to show any marked difference in behavior toward the parasite, 44 plants of Alaska and 34 plants of Rice's No. 330 were grown in uniformly inoculated soil at 27° C., the optimum temperature for infection. This experiment was started in a cloudy December when peas did not grow vigorously. Through an oversight the seed of No. 330 used was older than that of Alaska, a factor that may have given weaker plants of this variety. At any rate, the surviving Alaska plants grew more vigorously and gave more evidence of resistance than did those of No. 330. This experiment was repeated in January with the same result.

When the method which was used in these experiments is considered, it will be seen that although it secures results in a short time it is open to the objection that it subjects the germinating seed to higher soil temperatures than they ever encounter in the field, a condition which may reduce resistance. Accordingly a new series was started on the last day of February in which the soil temperature was maintained at 15° C. for two weeks before the temperature was raised to an optimum for infection. About 30 plants of each of the following varieties were grown from seed produced the previous summer:

Smooth peas—Alaska, Rice's No. 330; wrinkled peas—Rice's No. 13, Admirals (yellow), Eclipse, Horsford's Market Garden; Canada field peas—Scotch Beauty, Canada White.

One week after the plants were transferred to the higher soil temperature wilting began to appear, and on three sunny days half of the Eclipse, and a third of the Alaskas, yellow Admirals, and Scotch Beauties were thus destroyed. On March 21, when the Alaska plants were producing flower buds, all plants were washed from the soil and examined. Since there is much individual variation between plants of the same variety, an estimate of resistance is largely a personal judgment based upon a comparison of the damage that the plants have sustained and of their

vigor as follows: First, the extent of decay in the susceptible region above the attachment of the cotyledons; second, the extent of vascular discoloration above the region of cortical decay; third, the extent of damage that the taproot and rootlets have sustained; and, fourth, the vigor of root growth.

Alaska compared with No. 330 showed no difference in extent or character of injury. All plants were so badly decayed that seed production was not likely. All varieties suffered approximately equally in the susceptible zone at the base of the stem and in the extent of vascular discoloration above this region. The varieties of Canada field pea showed a more vigorous development of rootlets above this injured region, and these rootlets showed fewer lesions than were present on those of the canning varieties, whether of the starchy or wrinkled types. The extreme differences in root production between the field pea and canning types are shown in Plate 1.

A final comparison of Alaskas with No. 330 was started April 7 at a soil temperature of 27° C. from the time of planting. All plants were far more vigorous in the increased sunlight obtaining at this season. Very few plants of either variety wilted, but all were considerably stunted until May 1, when improvement in growth and color was noted. On May 15 the Alaskas were inferior in vigor to No. 330 (Plate 1, A) and a comparison of the root systems when washed from the soil showed greater difference than that shown by the tops. Although the only living roots were those emerging from above the decayed stem bases, these were far more extensive and freer from injury on the No. 330 than on the Alaska plants.

The evidence contained in these experiments, limited though it is, indicates clearly that among the varieties of peas tested, there is no conspicuous degree of resistance to decay at the base of the stem. The plants which are able to develop in spite of this decay, do so by virtue of the vigor with which they can send out new roots above the point of injury, and perhaps to some degree to the resistance of these rootlets to destruction by the fungus which may be combined with resistance of the vascular system to invasion. The susceptible region at the base of the stem does not appear to become more resistant as the plant grows older, though the damage to this region is less serious to an older plant. These experiments also suggest that an accurate control of soil conditions is not sufficient in comparing varieties of peas for resistance, but that intensity and duration of illumination which indirectly affect the vigor of root growth may also affect resistance. Further study of resistance was not made because it became clear that even should a far greater degree of resistance to this fungus be found, only a small degree of progress would be made in finding a plant that would survive in most diseased fields, provided resistance to this fungus was not coupled with resistance to other more important diseases.

PATHOGENICITY OF SPECIES OF FUSARIUM ISOLATED FROM VASCULAR BUNDLES OF DISEASED PEAS

During two summers great numbers of isolations were made of fungi present in discolored vascular bundles of diseased pea plants in order to determine whether any among these fungi which certainly contribute toward the destruction of plants are capable of acting as primary parasites. Material was collected largely in Wisconsin, with many representa-

ive collections from other States. A large majority of cultures obtained were species of Fusarium which upon classification were found to consist of five or six species in almost equal numbers, any one of which was obtained as frequently as the parasite previously described. From this collection the following species were selected for thorough trial of pathogenicity, some because of the frequency of their occurrence, and others because of previous mention in literature as parasites: *Fusarium oxysporum* Schlecht., *F. solani* Mart., *F. sclerotioides* Sherb., *F. vasinfectum* Atk., *F. redolens* Wr.

FUSARIUM OXYSPORUM Schlecht.—This species was given a most thorough trial, not only in the field, but in soil held at the entire range of temperatures used in the experiments previously noted. In all cases the plants remained as healthy as the controls. No evidence of parasitism was obtained.

FUSARIUM SOLANI Mart.—Cultures of this fungus were obtained more frequently in early spring. After unsuccessful attempts to produce infection at greenhouse temperatures, sterile soil was inoculated with the fungus and held at 14°, 18°, 22°, and 26° C. At 18° C., 6 plants out of 9 showed a slight superficial browning of the base of the stem. At 22° C., 8 plants among 10 showed a similar browning but deeper. The fungus was recovered from these lesions. At 26° C. the stems were not injured, but a considerable number of dead rootlets were found. Thus this fungus can be regarded as a very weak parasite at a temperature somewhat lower than that required by the other species tried, but probably not of economic consequence.

FUSARIUM SCLEROTIOIDES Sherb.—Although this fungus was isolated more frequently than any other, inoculations in greenhouse and field from cultures obtained during the first summer of work gave no positive indications of pathogenicity. A heavy inoculation of soil with spores from a culture obtained the following year produced a wilt in three plants when the soil was held at 27° C., 25 days after planting. The fungus was reisolated from the discolored vascular strands. Ten out of 16 remaining plants at this temperature showed discoloration of base of stem, and much bronzing of small roots. When peas were replanted in this soil the resulting injury was much less. Thus, although this fungus can be a parasite under conditions favorable for infection, it does not appear that it is ever an important parasite under field conditions.

FUSARIUM VASINFECTUM Atk.—The variety *odoratum* was found among the cultures, but was not used in making inoculations. In a series parallel with that described for *F. solani* no infection was obtained. Field inoculations gave no evidence of infection. The evidence does not indicate that this fungus can gain unaided entrance to the vascular bundles where it is so often found.

FUSARIUM REDOLENS Wr.—Although this fungus has been mentioned by Wollenweber as a vascular parasite of peas, the two cultures obtained by the writer gave no more than a trace of infection from which the fungus could not be reisolated.

Among all the cultures of Fusarium obtained from peas, no cultures of the *Fusarium falcatum* reputed to be the cause of the St. John's disease in Holland, or of the *F. viticola* found by Turesson in Sweden were obtained. A culture of *F. falcatum* obtained from another source gave no infection. The only species of this genus that has been found able to enter an uninjured pea plant to produce appreciable damage is the

species described in the body of this paper. There are, however, a number of species that enter the vascular bundles of peas when they have been exposed by other agencies of decay, and once within may hasten greatly the destruction of the plant. These species are not to be regarded as parasites, inasmuch as they do not penetrate and destroy living cells.

SUMMARY

1. A very destructive stem and rootrot of peas is known in almost all regions where peas have been grown for a long time. Investigators who have studied this disease have usually found a species of Fusarium to be the cause of decay, but in different parts of Europe and of the United States different species have been described as the parasites. The present investigation, which has extended over four years, has discovered but a single species of Fusarium parasitic upon peas in the United States. This species is that previously reported by Bisby in Minnesota, and it is here described as *Fusarium martii* var. *pisi*. Other species of Fusarium are sometimes found, sometimes consistently during a season in a locality, in the vascular bundles of peas, but they are found to have gained entrance not by traversing or destroying the living cells surrounding the vascular system, but through cells which have been killed by some other invader. A few other species of Fusarium are found able to enter and destroy a limited amount of parenchyma in a susceptible region at the base of the stem under very favorable conditions of temperature; but they are not regarded as important parasites under field conditions.

2. The species of Fusarium described here is found widely distributed in pea-growing districts of the United States; but the injury which it causes is far less important than that caused by Aphanomyces sp.

3. Several species of Pisum are susceptible to the disease.

4. The most susceptible portion of the plant is the base of the stem just above the attachment of the seed. The fungus entering here in seedlings may invade the vascular system and produce a wilt, but older plants are rarely so affected. Small rootlets are invaded, especially at the growing points and killed.

5. A comparatively high soil temperature, above 18° C., favors rapid development of the disease; but variations in soil moisture within the limits favorable for plant growth do not appear to affect its development.

6. Soils containing much organic matter appear to favor the persistance of the fungus in the field.

7. No evidence of dissemination of the fungus by seed has been obtained. Its wide distribution and the variability in the pathogenicity of cultures indicate that it is a widely disseminated soil organism having physiological varieties capable of varying degrees of pathogenicity.

8. A number of selected varieties of peas have been grown in infested soil which has been held under uniform controlled conditions to determine possible differences in resistance to disease. No marked differences in the resistance of the susceptible cortex at the base of the stem has been found. However, the vascular tissue beneath the parenchyma seems more resistant to invasion in certain varieties. There is an apparent difference in the resistance of the small rootlets to injury, and varieties capable of rapid extension of the root system possess an apparent resistance. However, a greater degree of resistance than has been indicated by this work must be found in desirable varieties before it can be regarded as of commercial importance, unless such resistance can be combined

ith resistance to the more important Phycomycetous parasites, which ill be discussed in a later paper.

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PLATE I

A.—Comparative resistance of Rice's No. 330 to *Fusarium martii pisi* under optimum soil conditions for infection compared with Alaska. Left, two cans of Rice's No. 330 grown at 27° C. soil temperature in infested soil in April and May. Right, two cans of Alaskas grown under identical conditions. In uninfected soil these varieties make approximately equal growth. In this case the Alaskas are not only dwarfed, but have many dead and yellowed leaves at the base of the stems.

B.—Degrees of stem and root injury caused to bases of stem and roots of peas by *F. martii pisi*.

Left, stem completely rotted off from taproot at seed. The plant is supported by a few new roots arising from upper portion of the underground stem. Variety, Alaska.

Center, stem connected with taproot only by an exposed vascular strand. Branches of the taproot nearly dead. Variety, Alaska.

Right, stem connected with taproot by exposed vascular strand. Branches of taproot still alive. Vigorous production of new roots from above the point of injury. Variety, White Canada.


A
B

HORNWORM SEPTICEMIA [1]

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INTRODUCTION

The larvæ of two species of insects, *Protoparce sexta* Johan. and *Protoparce quinquemaculata* Haw., of the family Sphingidae, are frequently called hornworms, a name suggested by a large, curved, hornlike spine on the dorsal and posterior portion of the body. They are the large, naked, green "worms" [2] which feed so greedily on the leaves of growing tobacco and are then commonly called tobacco worms. When they feed on the tomato plant they are often referred to as tomato worms. The potato and eggplant also furnish food for them.

A disease sometimes occurs among hornworms. The remains of the larvæ that die of the disorder darken soon after death and as a result of drying become a black, mummified, shriveled mass. During the course of the disease in these larvæ a bacterium may enter the blood stream and multiply rapidly therein to a marked degree. This septicemia is one of the most pronounced characters of the disorder and suggested the name "hornworm septicemia" which is here given to the disease.

Very little literature is found regarding the diseases of hornworms, and at the present time it is not possible to know definitely whether the existence of the disorder described in this paper has heretofore been recorded. Garman (*1, p. 30*) [3] in 1897 states that he has:

> Sometimes observed dead and blackened worms clinging to the plants, head down, by means of the hooks on their fleshy legs, * * *.

The presumption indicated by him, however, is that they had died of a fungous infection, and he cites observations made by Thaxter (*6, p. 96*) in 1890. Lovett (*4, p. 171*) states that:

> A bacterial disease sometimes attacks these worms, causing them to shrivel up, turn black, and die.

A similar statement is made by Reed (*5, p. 26*).

A. C. Morgan and his coworkers at the tobacco insect laboratory of Southern Field Crop Insect Investigations, Bureau of Entomology, at Clarksville, Tenn., encountered this disease and observed that it could be transmitted to healthy larvæ by the puncture method of inoculation. In a letter Mr. Morgan writes:

> In 1917 some special experiments with nearly mature hornworm larvæ were ruined because of the rapid spread of hornworm septicemia in the experimental cages. Since that year no great numbers of larvæ have been under observation at any one time except in hibernation cages. Although the disease has always been in evidence in the hibernation cages, yet it has never been sufficiently severe seriously to affect the experiments. I do not believe that this disease is of much economic importance in this region, for diseased larvæ are rare under natural conditions.

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[1] Accepted for publication October 2, 1923.

[2] For convenience the term "worms" is used frequently in this paper as an abbreviation of "hornworms."

[3] Reference is made by number (italic) to "Literature cited," p. 486.

In September, 1917, the writer began experimental studies on this disorder and these were continued with many interruptions until the present time (1922). The disease material used in the work was received from the laboratory at Clarksville.

Hornworm septicemia is of much interest, first, because it is a disease of two species of insects which are of great economic importance; and second, because it belongs to the large and important group of insect diseases of which the much discussed *coccobacillus* infection in grasshoppers is a member. Much is yet to be learned about this disorder, but the facts already obtained and given in the present paper will suffice to answer many questions likely to be asked concerning it.

SYMPTOMS AND POST-MORTEM CHANGES

The infected larvæ lose their appetite. Their normal stool of berry-like pellets changes in the infected larvæ to a semifluid one and then to a watery discharge. This dysenteric condition is one of the prominent signs of the disorder. Late in the course of the disease a thin "vomitus" oozes from the mouth. The pronounced turgidity seen in healthy worms becomes lost in the infected ones.

A larva dead in the experimental cage following inoculation is usually found lying on its side occupying a slightly curved position. The remains of the larva that has died on the growing plant are found hanging usually head downward by means of the hooks of a proleg (Pl. 1, A, B). The semifluid body content gravitates cephalad in this position.

Soon after death the body of the worm becomes light brown, deepens rapidly to a dark shade, and finally turns almost black. The body wall at first resists puncture and tearing quite as much as during life, but later is more easily ruptured. The tissues within undergo a rapid change, becoming soon a brown semifluid mass in which silvery white portions of tracheæ are seen.

The body wall remains intact if the decaying larva is undisturbed. When drying takes place, the remains diminish in size but retain in general the larval form, becoming in a week or so, depending much on the climatic conditions, a dry, shriveled, friable, dark brown to black mass.

EXCITING CAUSE OF THE DISEASE

The media and methods commonly used in the laboratory are sufficient for the culture work. In the experimental inoculations the two methods not infrequently employed in insect studies have been followed. These may be designated as the puncture method and the feeding method. By the puncture method the body wall is pierced by a fine dissecting needle which is first sterilized by flaming and cooled, and the point of which is then contaminated by thrusting it into the tissues of the sick or recently dead larva or by dipping it into a culture, an agar one being most frequently used. Any convenient place on the body of the larva may be chosen for the puncture, the intersegmental spaces being as a rule the easiest to pierce. No attempt need be made to sterilize the area at the point of inoculation. The small amount of blood which the insect loses causes it no particular inconvenience. Control larvæ punctured with a sterile needle manifest no ill results from such a treatment. Likewise a larva punctured by a needle which has been dipped into the blood of another healthy larva or into unsterilized tap water suffers no

nfection therefrom. By the feeding method the leaves given the larvæ as food are first dipped into an aqueous suspension of the culture or of the tissues of worms sick or recently dead of the disease.

From a study of the blood of sick worms and of those dead of the disease a bacillus was found in very large numbers and in pure or almost pure cultures. When healthy worms are inoculated with a pure culture of this bacillus by the puncture method the mortality is 100 per cent. The sick ones show symptoms and the dead ones post-mortem changes that are similar to those observed in worms which become infected in nature. In microtome sections of sick worms one sees further proof that the condition is a septicemia, the bacilli being found throughout the blood spaces. They appear particularly numerous [4] between the folds of the stomach wall.

The name *Bacillus sphingidis* [5] is here used for the bacterium which has been encountered in this disease.

Bacillus sphingidis n. sp.

This species grows readily in all of the common and differential media ordinarily employed. Good growth is obtained in media whose reaction varies from +1.5 to

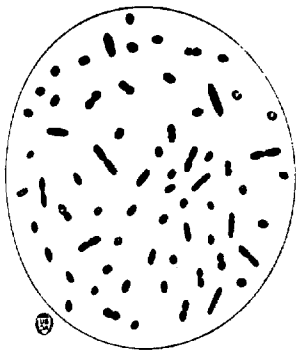

FIG. 1.—*Bacillus sphingidis.*

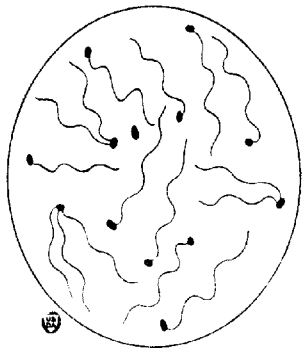

FIG. 2.—The flagella of *Bacillus sphingidis.*

−2 per cent, and occurs also 1 per cent or more beyond these limits, but it is then less rapid and less extensive. For incubation a wide range of temperature is suitable. That of the room is very satisfactory and was used for most of the work.

MORPHOLOGY.—The organisms of a 24-hour plain agar culture are small, short rods with rounded ends, many forms being coccoid and ovoid; the coccuslike ones measure about 0.6 micron in diameter and the ovoid ones about 0.5 by 0.75 micron. (Fig. 1.) The forms which are distinctly rod-shaped are from 0.8 to 1.5 microns in length and from 0.5 to 0.7 micron in width. In bouillon cultures the rod forms predominate and average larger than those from agar. Long rods and even filaments not infrequently occur in older bouillon cultures. No spores are produced. The small forms on agar have most frequently one flagellum each, although sometimes two and occasionally more (fig. 2) are possessed by a single bacterium. They are usually attached near an end of the rod.

MOTILITY.—The bacillus is actively motile. The movements, aside from being progressive, are usually decidedly whirling.

STAINING PROPERTIES.—It stains easily and uniformly with the usual aniline dyes and is gram-negative.

[4] That some of the bacilli escape from the blood to the alimentary canal is shown by their presence in large numbers, in worms inoculated by puncture, in the thin alvine discharges that occur during the latter part of the course of the disease, but the mode of exit from the blood has not been observed. The larger number in the blood spaces between the folds of the stomach wall may be partly, or indeed wholly, due to the technique used in the preparation of the slides.

[5] This name was suggested by Dr. L. O. Howard.

AGAR PLATES.—The surface colonies on plain agar form rapidly, measuring 1 mm. within 24 hours and 5 mm. within 1 week. The outline is circular and entire, and the surface is oval and glistening. By reflected light the growth is gray with an opaque center becoming translucent toward the border; by transmitted light it is bluish gray. Magnified, the colony is brownish with a more or less uniformly granular structure. The bacterial mass is nonviscid and adheres to the medium.

GELATIN PLATES.—In 24 hours at room temperature the colony is surrounded by a small area of liquefaction.

AGAR SLANT.—A slightly raised, bluish gray, friable growth of moderate amount and limited to the surface inoculated is present in 24 hours. Old cultures show a light yellowish brown tint.

GELATIN STAB.—At room temperature in 24 hours there is a moderate white growth along the line of puncture with beginning liquefaction. This proceeds slowly, being greater at the surface, becomes crateriform to infundibuliform, and is complete within three weeks. At lower temperatures the growth and liquefaction are slower.

POTATO.—In one day there is a feeble, moist, slightly yellowish growth which increases and becomes medium in quantity later and of a brownish hue. The potato is discolored.

BOUILLON.—Growth takes place rapidly, becoming slightly clouded in 4 hours and turbid in 24. A very thin, delicate pellicle may form and much friable sediment is present in old cultures. The medium remains clouded.

MILK.—No change is apparent during the first two days. A coagulum is present on the third day; by the end of a week 1 cm. of this is digested; and by the end of one month one-half is dissolved, leaving a brownish, turbid, slightly viscid whey.

LITMUS MILK.—The medium appears unchanged on the first day, becomes alkaline on the second, and continues to be so.

CARBOHYDRATES.—A carbohydrate as a rule enriches a medium. Fermentation with acid occurs in dextrose, levulose, galactose, mannose, maltose, saccharose, lactose, glycerin, mannite, arabinose, xylose, dextrin, and in the glucoside salicin. From lactose and arabinose only a slight amount of acid was produced within 24 hours, the medium changing to alkaline during the second or third day. No acid was formed in raffinose, inulin, and erythrite. Gas is produced in small quantities in glucose, levulose, mannose, saccharose, salicin, and possibly occasionally in other media.

SUGAR-FREE BOUILLON.—No indol is produced.

BLOOD-AGAR PLATES.—No hemolysis occurs on blood agar made from whole rabbit's blood.

FURTHER EXPERIMENTAL INOCULATION

A variety of preliminary inoculation experiments were performed to determine further the nature of hornworm septicemia. Some of these are summarized here. In the following group hornworms were used and the inoculations were made by puncture: In 12 experiments, 76 larvæ were inoculated using the tissues of worms dead less than 24 hours of hornworm septicemia. All of these died of the disease. In two experiments, 15 larvæ were inoculated with disease material from worms which had been dead two or three days. Only one of these became infected and died. In four experiments 34 larvæ were inoculated using a pure culture of *Bacillus sphingidis*. Of these 33 became infected and died.

In another group the feeding method of inoculation was employed. In two experiments using 34 larvæ, the disease material was obtained from worms dead less than one day of hornworm septicemia. Of these 6 died of the disease. In two experiments in which 12 larvæ were used worms dead from two to five days furnishing the disease material, none died. In two experiments 20 larvæ were fed pure cultures of *Bacillus sphingidis* and of these 2 died of hornworm septicemia. In two experiments, the body wall of the 20 larvæ used was first pierced in several places with a sterile needle and then the worms were fed leaves which had been dipped into an aqueous suspension of crushed tissues of worms

ecently dead of the disease. None of these larvæ died, indicating that nfection in nature probably does not take place through preformed brasions in the body wall. In two experiments in which 19 larvæ were sed the cages in which they were placed had been heavily contaminated ith disease material from larvæ recently dead of the disease. Of these orms, 2 died.

In four cages used as controls there were 32 larvæ. All of these com- pleted their feeding period without becoming diseased. Among the ery large number of worms collected by the men at the Clarksville aboratory the disease was rarely encountered. In cages in which a rge number were kept for a few days only occasionally a worm was ound dead of the disorder. These cages, therefore, also served as controls. As a further control 27 larvæ in five experiments were fed leaves which had been immersed in aqueous suspensions of decaying larvæ that were not dead of hornworm septicemia. In this material many unidentified bacteria were present, most of them being probably of post-mortem origin. None of these control worms died of the disease.

RESISTANCE AND VIABILITY

Bacillus sphingidis from a two-day bouillon culture is killed in a 2 per cent aqueous solution of carbolic acid in less than one-half minute; in a 1.5 per cent solution, in about two and one-half minutes; in a 1 per cent solution, in about five minutes; in a 0.75 per cent solution, in about one hour; in a 0.5 per cent solution, in about two hours; while a 0.25 per cent solution permits a feeble growth of the species.

A three-day agar culture of *Bacillus sphingidis* suspended in normal salt solution, sealed in an ampule and immersed in a water bath, is killed in 10 minutes at a temperature between 53° and 54° C. Suspended in tap water, which is allowed to evaporate, the organisms in the film are dead in less than one day after becoming dry. Sterilized sand to which an aqueous suspension of the culture was added was found to be practically sterile again after becoming dry, but the bacilli remained alive as long as the sand was kept moist. The latter tests, however, were continued for three months only. An aqueous suspension from an agar culture exposed to the direct rays of the sun was destroyed in from three to five hours. The bacillus in a similar suspension added to sand and exposed to the sun was killed in less than three hours.[6]

The culture remains alive on agar and in other media over long periods, dying out, however, when the medium becomes dry. Sealed agar cultures have remained alive at room temperature more than a year without renewal and probably will be found viable after a much longer period. Good growth has been obtained from four-month liquid cultures, and it is probable that this can be repeated from much older ones if drying of the media is prevented.

A bacteriological examination of the blood of larvæ just dead of the disease shows the presence of *Bacillus sphingidis* in very large numbers and in almost pure cultures. After a day or so following the death of the worms, however, the number of organisms in the remains diminishes rapidly. The decaying mass contains few viable organisms by the time it is dry.

[6] The temperature acquired by the sand may have been a factor in the destruction of the organism in this instance, and the drying a decided factor.

What seems to be a phagocytosis occurs in the infected worms. The presence of this phenomenon is suggested by the occurrence of groups, here and there in the blood spaces, of closely packed cells, which are not unlike those observed in infections in other insects. At summer temperature at least the protection received in consequence of this and what other protective phenomena the larva may possess is, however, wholly inadequate to preserve the life of worms inoculated with *Bacillus sphingidis* by the puncture method.

PATHOGENESIS

Hornworms in all instars are very susceptible to infection with pure cultures of *Bacillus sphingidis* when the puncture method of inoculation is followed. Larvæ in the fifth instar, this stage being used most often for experimental purposes, become infected and die in about 100 per cent of the inoculations. When the feeding method is employed, however, a relatively small percentage of the worms die.

The period from the inoculation of a hornworm by puncture to its death varies considerably, depending chiefly on temperature. During the warmer days of summer, death takes place in one day or less, while this period is more often extended to two days or more during the cooler weather. At incubator temperature it may be less than twelve hours.

The susceptibility of the larvæ of the silkworm (*Bombyx mori* L.) to experimental infection with *Bacillus sphingidis* is about equal to that of hornworms (Pl. 1, F). The same is found to be true of the larvæ of the catalpa moth, *Ceratomia catalpae* Bdv. (pl. 1, E). Cutworms inoculated died in abou tthree days (pl. 1, C). Grasshoppers also are susceptible to experimental infection (pl. 1, D). All of these insects except the hornworms and silkworms were tested by the puncture method alone. No species was found to be immune to puncture inoculations.

Silkworms were inoculated each year from 1918 to 1921, inclusive, with a culture of *Bacillus sphingidis*, isolated in 1917 and kept on agar at room temperature, and no evidence of any change in virulence was observed By puncture inoculation the culture produced septicemia and death in hornworms as readily in 1921 as in 1917, when it was first isolated. The virus direct from the decaying tissues of recently dead worms seems to kill in slightly less time than do isolated cultures of the organism.

A rabbit inoculated intravenously with pure cultures of the bacillus showed on the following day a tendency to anorexia, but soon recovered and for a month thereafter no further symptoms were noted. An autopsy on the etherized animal showed no lesions of note.

COMPARISON OF BACILLUS SPHINGIDIS AND B. ACRIDIORUM

One of the specially interesting facts brought out by the study of the diseases of insects is that among them there is a large group in which a true septicemia occurs, the infecting organisms being in many respects similar. They are actively motile, gas-producing, and nonsporulating short bacilli which are often coccoid in form when grown on solid media. In the literature these have frequently been referred to as coccobacilli. Beginning about 1911, the study of this group was given a marked impetus by the work of the French investigator d'Herelle (3) on an infection in grasshoppers. D'Herelle encountered and described as the cause of the

disease a bacillus to which he gave the name *Coccobacillus acridiorum*. Glaser (*2*) made a study of a number of cultures which had been isolated and identified as *Bacillus* [7] (*Coccobacillus*) *acridiorum* and found a considerable variation among them. Of those he compared, two were received from d'Herelle designated as "souche sidi" and "souche cham," respectively. Some differences were noted in these two also. The two from d'Herelle were obtained by the writer from Dr. Glaser in order that *B. sphingidis* might be compared with them. It was soon demonstrated that *B. acridiorum* and *B. sphingidis* were related species and should be placed in the same group of organisms.

The serum of a rabbit [8] immunized with *Bacillus sphingidis* and showing an agglutinin titer of 1:4,000 for the culture of *B. sphingidis*, used in the immunization, did not agglutinate either the "souche sidi" or "souche cham" strain of *B. acridiorum*. Hornworms, silkworms, and the larvæ of the catalpa moth were killed in slightly less time from puncture inoculation with *B. sphingidis* than with *B. acridiorum*.

PREDISPOSING CAUSES

Little is known concerning the predisposing causes of hornworm septicemia but it seems quite certain that there are important contributing factors besides the exciting agent. From the foregoing pages it is seen that a septicemia and the death of the worm follow readily the introduction of *Bacillus sphingidis* into the blood by puncture inoculations. If in nature the organism reaches the blood through the chitin-covered body wall, such entrance, it would seem, must be accompanied by an abrasion of the wall, the introduction of the germ, if it occurs at all, being more likely at the time of the trauma.

If the portal of entry of *Bacillus sphingidis* in the production of the septicemia is by way of the alimentary tract, as seems quite probable, evidently there are here also some very effective protective forces of the host which must be overcome before the bacillus is able to gain entrance to the blood. What these are is another interesting problem only partially solved.

The age of the larva seems to be one of the predisposing causes, infection being more likely to occur during the fifth instar. Temperature may be another, warm weather seeming to predispose the larva to the septicemia. Differences in susceptibility before, at, or following the molting period, if indeed there are any such, have not been determined.

[7] Inasmuch as Coccobacillus as a generic name does not occur in systems of classification ordinarily followed by American writers, Bacillus, more generally employed, is used in the present paper.

[8] The rabbit was injected intravenously with 1 cc. of a normal salt suspension of *Bacillus sphingidis* made from a 24-hour agar culture, containing about 100 million organisms. Similar injections were repeated weekly until five of them had been made, using each successive time a like suspension but of increased density. The rabbit was bled one week following the last injection.

A two-day bouillon culture was found to be a very suitable one for making the agglutination test. This was diluted to the desired density with a one-half of 1 per cent solution of carbolic acid in normal salt. The macroscopic method was followed using room temperature. The clumping was evident within a short time, but the final reading was usually made after the tubes had stood overnight.

The agglutination test with this species is very satisfactory, the positive tubes clearing completely while the control suspension remains uniformly clouded with no tendency to clump and with only a slight tendency to settle. There is no agglutination with normal rabbit serum. Agar and old bouillon cultures may be used but were found to be less desirable than the two-day bouillon ones. The test was satisfactory also when a one-half of 1 to 1 per cent carbolized suspension was used, but when a 2 per cent one was employed it was apparently somewhat impaired. Bouillon cultures when heated to 65° C. for 10 minutes proved to be as useful in the test as the carbolized unheated ones. When heated to 80° C., however, their agglutinability was somewhat impaired and when boiled for 10 minutes it was destroyed.

The immune serum, after being drawn, was diluted 1:3 with normal salt solution carbolized to one-half of 1 per cent, sealed in glass ampules, and kept at room temperature shielded from light. Tested after more than two and one-half years the agglutinating power of the serum was practically unimpaired, but when tested still a year later this property was found to be virtually lost.

Likewise any differences in susceptibility that might exist in the two species of hornworms referred to in the present paper are as yet unknown.

Summarizing the causes of hornworm septicemia, one finds that *Bacillus sphingidis* is the infecting organism and the immediate cause of the death of the worms. There also are predisposing causes which are evidently very important but which are as yet largely undetermined.

TRANSMISSION OF THE DISEASE

Little is known of the distribution of *Bacillus sphingidis*. The ease with which this germ is destroyed through drying and its low pathogenesis in nature would suggest that hornworm septicemia with the death of large numbers of the worms, especially during the active growing season of the tobacco and tomato crops, is not likely to occur. The field observations indicate that the disease under these conditions does not spread readily and that a wholesale destruction of the worms does not take place. When large numbers of worms have been kept together in cages for a few days occasionally an infected one has been found among them. Little is known of what occurs in this connection during hibernation.

From what is known of hornworm septicemia, its exciting and predisposing causes, its pathogenesis, and its modes of transmission, the artificial use of the disease to control the losses due to the feeding larvæ would not at the present time seem to be a justifiable economic procedure.

DIAGNOSIS, PROGNOSIS, AND TREATMENT

A provisional diagnosis of hornworm septicemia is justified from the symptoms and post-mortem appearances of the disease, but a positive one can be made only by demonstrating the presence of *Bacillus sphingidis* in the sick or recently dead worms. In making the diagnosis healthy worms may be inoculated by puncture, using the tissues of larvæ sick or recently dead of the disease, and if symptoms of hornworm septicemia are produced and death with post-mortem changes characteristic of the disorder occur the disease may be strongly suspected, the diagnosis being confirmed by finding the causal organism of the septicemia.

Worms which meet death simply through violence do not as a rule undergo post-mortem changes present in hornworm septicemia. Worms dead of poisoning were shown the writer by one of the men at the Clarksville laboratory, which were accompanied by post-mortem appearances not unlike those accompanying this hornworm disease. Upon examination, however, it was found that *Bacillus sphingidis* was not present, showing that hornworm septicemia was not the cause of death. Experimental infections by some species other than *B. sphingidis* are followed by death and post-mortem changes quite similar to those in hornworm septicemia. Worms dead from parasitism with *Apanteles congregatus* Say have been found hanging by a proleg and discolored like those dead from infection with *B. sphingidis*. All of these conditions must be differentiated from the hornworm disease.

When septicemia occurs in an infection with *B. sphingidis* death is almost inevitable, if not entirely so. Nothing is known definitely about the disease condition in the body, prior to the invasion of the blood stream by the infecting organism. Since only a small percentage of worms inoculated by the feeding method die, it is not improbable that in this disease some of them suffer an abnormal condition within the alimentary tract, from which they may recover.

A treatment of hornworm septicemia would be of interest especially to those who rear the worms for study. Preventive measures are suggested as the most promising. The facts recorded in the present paper may aid in devising such means.

SUMMARY AND CONCLUSIONS

(1) A disease is occasionally encountered among larvæ of *Protoparce sexta* and *P. quinquemaculata* of the family Sphingidae in which death is preceded by a marked septicemia and followed by a dark and almost black discoloration of the remains.

(2) The name hornworm septicemia is here suggested and used for this disorder.

(3) The disease in the worms inoculated by puncture runs a course of from 18 hours to 2 or 3 days in which the most prominent symptoms are loss of appetite, stupor, diarrhea, and a thin vomitus. The more important post-mortem changes are a softening and blackening of the remains, which on drying become shriveled.

(4) The organism of the bacteriemia is a short, actively motile, non-sporulating bacillus to which the name *Bacillus sphingidis* is given.

(5) The bacillus is readily destroyed by heat, drying, direct sunlight, and chemical disinfectants, but lives a long period in a moist environment at room temperature.

(6) A comparatively small percentage of healthy worms die following inoculation with the virus of the disease by the feeding method, but practically 100 per cent of them succumb following puncture inoculations.

(7) Cutworms, catalpa-moth larvæ, and grasshoppers are very susceptible to puncture inoculations with *Bacillus sphingidis* and die speedily from septicemia. Indeed no insect species thus inoculated has been found immune.

(8) No appreciable loss of virulence has been noted in cultures of this bacillus kept four years on artificial media.

(9) *Bacillus sphingidis* is similar in many respects to *B. (Coccobacillus) acridiorum* d'Herelle, the cause of a grasshopper disease discussed by d'Herelle. They show, however, a distinct serological difference.

(10) The transmission of the disease in nature probably takes place as a rule by way of the alimentary tract, the portal of entry of the germ not being definitely known.

(11) The diagnosis of hornworm septicemia is suggested by the symptoms and post-mortem appearances and can be made positive by the isolation of *Bacillus sphingidis* from the sick larvæ or from the remains of those recently dead.

(12) Apparently comparatively few hornworms die of the disease in nature during the more active growing season of the crops on which these worms feed.

(13) Preventive methods are recommended to students of hornworms who may desire a treatment for this disease. Facts given in the present papers will serve as a guide in devising such means.

(14) There is need for a much more comprehensive study of the group of insect diseases of which hornworm septicemia is a member, and the group of bacteria to which *Bacillus sphingidis* belongs. It is hoped that the facts given in the present paper will be useful in answering many

questions likely to arise in connection with this hornworm disease and this group of diseases in general.

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PLATE 1

Hornworm septicemia:

Inoculations with *Bacillus sphingidis*, using the puncture method. All of the insects were dead. Photographed and reproduced at about natural size.

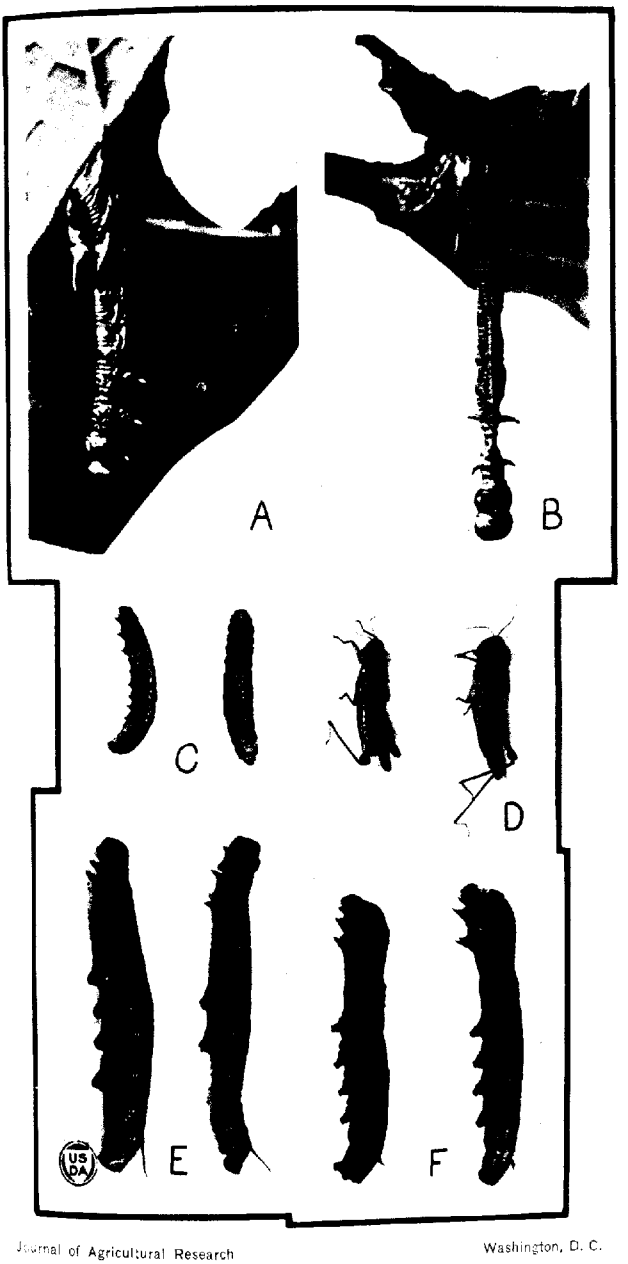
A and B.—Hornworms two days after inoculation hanging by hook of proleg from leaf of tobacco plant on which they had been feeding.

C.—Cutworms three days following inoculation.

D.—Grasshoppers two days following inoculation.

E.—Catalpa-moth larvæ two days after inoculation.

F.—Silkworms, fifth instar, two days after inoculation.


A
B
C
D
E
F

CUTWORM SEPTICEMIA[1]

By G. F. WHITE

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INTRODUCTION

Farmers are well aware that cutworms exact annually a considerable toll from their crops. These pests are the larvæ of numerous species of moths belonging to the family Noctuidae. The mature "worms" [2] are naked, plump, and of medium size. Their color and markings vary greatly. Those attacking the young plants of gardens and fields are frequently grayish or brownish with spots or linear stripes; and those which prefer to feed on nursery stock and young orchards are of a light yellowish gray.

Entomologists have observed that sometimes cutworms die from disease, their remains becoming soft and assuming a dark color. In 1899 Cavara [3] in Italy recorded the presence of a disease of *Agrotis aquilina* Hb. in which the larvæ dead of the disorder turn a chestnut brown and become mummified and fragile. He found in the dead remains a bacterium in huge numbers which grew rapidly on gelatin at room temperature and quickly liquefied the medium. The bacterium is described as a rod with rounded ends measuring from 1 to 1.5 microns and resembling at times a diplococcus but occurring also in long chains. No name was suggested for the species. He observed also that when the larva of *Hylotoma pagana* Panz. was inoculated with a culture of the bacterium by puncture, death resulted in a very short time, but when the feeding method of inoculation was employed the larva did not die. He suggested the use of the disease as a possible artificial means for the control of *Agrotis aquilina* in parts of Italy where this cutworm was particularly numerous and destructive. The observations by Cavara are interesting but his description is not sufficient to make it possible to state whether the disorder observed by him is the one discussed in the present paper.

In 1917 S. E. Crumb, working in the laboratory of Southern Field Crop Insect Investigations of the Bureau of Entomology at Clarksville, Tenn., encountered a disease among cutworms in which the remains of the dead worms became soft and turned dark in color. He demonstrated that the disease could be transmitted to healthy worms by puncture, using the tissues of larvæ dead of the disease in making the inoculations.

In September, 1917, the writer began a study of the disorder encountered by Mr. Crumb, using material furnished by him. It was found that a marked septicemia is present in larvæ showing symptoms of the disease. This observation suggested the name "cutworm septicemia" which is here used for the disorder. The disease is similar in many ways to hornworm septicemia described in the preceding paper.[4]

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[1] Accepted for publication October 2, 1923.

[2] The term "worms" used in this paper is an abbreviation of cutworms.

[3] CAVARA, F. DI DUE MICRORGANISMI UTILI PER L'AGRICOLTURA. *In* Bul. Soc. Bot. Ital., ann. 1899, p. 241-243. 1899. Review in Centbl. Bakt. [etc.], Abt. 2, Bd. 6, p. 91. 1900.

[4] See HORNWORM SEPTICEMIA, p. 477 this number, which will be found helpful in following the present paper.

Since cutworms are of great economic importance, naturally a disease of them is of special interest. Cutworm septicemia, moreover, belongs to the large and important group of diseases of which the coccobacillus infection in grasshoppers and a number of other diseases of insects already described are members. While there is much yet to be learned about cutworm septicemia, the facts already determined and contained in the present paper are sufficient to make possible answers to many of the questions likely to be asked regarding the disorder.

EXCITING CAUSE

The technique that was used in the study of hornworm septicemia is very similar to that which has been employed in the work on this cutworm infection. Both the puncture and the feeding method were employed in the inoculations. Before making the puncture no attempt need be made to sterilize the field of operation. Check larvæ punctured with a sterile needle, with one dipped into the blood of another healthy worm, or with one contaminated with unsterilized tap water suffer no infection or other particular inconvenience therefrom.

The blood of larvæ recently sick or dead of the disease is found to contain an actively motile bacillus in large numbers and in pure or practically pure cultures. Cutworms inoculated by puncture with a pure culture of the bacillus become infected, a pronounced septicemia results, and a mortality of about 100 per cent occurs. Cultures made from fecal and oral discharges from sick larvæ which had been inoculated by puncture contain the same bacillus that is present in the blood and in even greater numbers. The bacillus does pass, therefore, from the blood to the lumen of the alimentary tract, but its portal of escape is yet to be determined. Within the alimentary canal of the infected larva the bacillus apparently multiplies rapidly.

So far cutworms inoculated by feeding the bacillus have not shown symptoms of cutworm septicemia nor have they died from infection. On the other hand septicemia and death have followed the feeding of this germ to silkworms. If infection in cutworms can take place, as seems probable, through the ingestion of food contaminated with the bacillus, the portal of entry of the organism from the alimentary tract to the blood is yet to be discovered.

The presence of a true septicemia is shown also in microtome sections of sick larvæ infected by puncture, the organisms being found in all the blood spaces. The sections, furthermore, show the bacilli within the stomach, many not infrequently occupying a position near the epithelium of the organ.

The name *Bacillus noctuarum*[6] is used for the bacillus which is here shown to be present in the septicemia and the immediate cause of the death of the worm.

Bacillus noctuarum n. sp.

This species is a facultative anaërobe which grows very well on all of the common and differential media ordinarily used in the laboratory. Abundant growth is obtained in media varying in reaction from +1.5 to −2 per cent. Less extensive and slower growth is obtained from 1 to 2 per cent beyond these limits. Growth may be obtained within a considerable range of temperature. That of the room was found suitable and was used in most of the work here reported.

[6] The specific name of this bacillus was suggested by Dr. L. O. Howard.

MORPHOLOGY.—The rods in 24-hour agar cultures are so short that many of them resemble cocci (Pl. 1, B). Those which appear spherical measure about 0.6 micron in diameter, while others which appear ovoid are about 0.75 micron in length and about two-thirds as thick. Some of the rods are 1 micron or more in length and from 0.5 to 0.8 micron in thickness, while in older cultures longer rods and even filaments occur. In bouillon cultures the rods average larger, being both longer and thicker (fig. 1; Pl. 1, A). The shorter rods supplied with flagella possess most often one or two of them (fig. 2). Occasionally three or four are present, but rarely more. These spring from almost any part of the organism, but usually from near a pole. Spores are not produced.

MOTILITY.—The movements of the bacillus are both progressive and whirling.

STAINING PROPERTIES.—The rods stain readily with the usual anilin dyes and are Gram-negative.

AGAR PLATE.—The colonies on plain agar form rapidly and have a well defined, entire border and an oval, glistening surface. They are bluish by transmitted and grayish by reflected light. The colony structure is finely granular and more or less uniform. The growth is nonviscid and adheres to the medium.

GELATIN PLATE.—Within 24 hours a small area of liquefaction is present about the colony.

AGAR SLANT.—Within a day a moderate, bluish-gray growth occurs which is confined approximately to the surface inoculated.

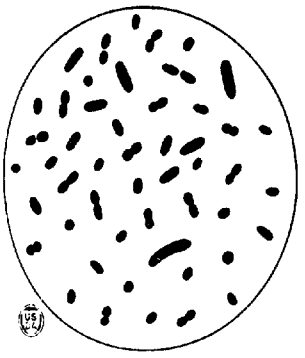


FIG. 1.—*Bacillus noctuarum.*

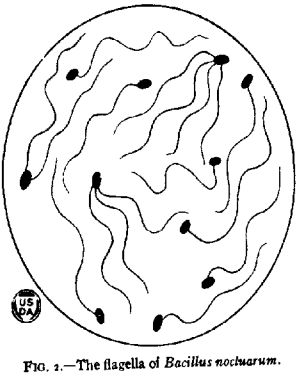


FIG. 2.—The flagella of *Bacillus noctuarum.*

GELATIN STAB.—In 24 hours at room temperature, a white growth is seen along the line of puncture, with beginning liquefaction along the entire needle tract. This is more marked near the surface of the medium. In three days the liquefied portion is infundibuliform and at the surface has reached the wall of the tube.

POTATO.—Within 24 hours a moderate, gray, moist growth occurs, which increases and becomes slightly yellowish. The potato becomes a grayish brown. Gas is formed.

BOUILLON.—Within four hours the medium becomes slightly cloudy and within a day turbid. It remains cloudy even in old cultures. A slight ring of growth adheres to the wall of the tube at the surface of the medium, a very delicate pellicle may be present, and a heavy friable sediment forms.

MILK.—Within a day a soft coagulum is present which is slowly digested, one-fourth being dissolved within a week and three-fourths within a month, leaving a yellowish whey.

LITMUS MILK.—Slight acidity is formed at first, which changes soon to alkalinity. The color is entirely discharged within a week.

CARBOHYDRATES.—In many instances the presence of a carbohydrate increases the luxuriance of the growth. Acid is produced in dextrose, levulose, galactose, mannose, saccharose, maltose, glycerin, mannite, xylose, dextrin, and salicin; very little is formed in lactose and arabinose; and none in raffinose, inulin, and erythrite. Gas is produced in small but visible quantities in glucose, levulose, mannose, saccharose, and salicin (Table I), and possibly occasionally in some of the other media.

SUGAR-FREE BOUILLON.—Indol is negative.

BLOOD AGAR PLATES.—Whole rabbit's blood is not hemolyzed.

RESISTANCE AND VIABILITY.—*Bacillus noctuarum* from a three-day bouillon culture is killed in a 2 per cent aqueous solution of carbolic acid in a few seconds; in a 1 per cent solution in two minutes; and in a 0.25 per cent solution growth takes place although retarded. The thermal death point in a similar culture is 54° C. exposed for 10 minutes. The bacillus in a film produced by the evaporation to dryness of an aqueous suspension taken from agar is found to be dead very soon after becoming dry. The organisms from a similar suspension added to sand soon die if the sand is allowed to become dry, but if it is kept moist they remain alive for a long period. Old cultures on agar or in liquid media remain viable until the medium becomes dry. After 15 months sealed agar cultures are alive and most likely will remain so very much longer if drying is prevented.

FURTHER EXPERIMENTAL INOCULATIONS

In seven experiments 20 cutworms of the genus Feltia were inoculated by puncture with material from worms sick or recently dead of cutworm septicemia. All of these died (pl. 2, A). Similarly 3 worms of the genus Agrotis and 2 of the genus Prodenia were inoculated and all of these also died. In the sick and dead worms *Bacillus noctuarum* was found in very large numbers in pure or nearly pure cultures. A pure culture was used in inoculating by puncture 8 cutworms in two experiments with the result that all of them died of septicemia.

In four experiments 12 hornworms were inoculated by puncture, using a pure culture of *Bacillus noctuarum*, and all of them died (pl. 2, M). Two of these worms kept at incubator temperature died within 20 hours. Likewise in seven experiments in which 20 silkworms were inoculated all of them died (pl. 2, I). A pure culture was used also in the inoculation of 8 catalpa moth larvæ and all of these died within one day (pl. 2, J).

Six cutworms were inoculated by the feeding method, using the tissues of worms sick or recently dead of the disease. None of them showed symptoms of infection or died of the disease. In two experiments 37 silkworms in the fifth instar were fed leaves immersed in an aqueous suspension of a culture of *Bacillus noctuarum*, and of these 24 died, the maximum temperature at the time of the experiment being 34° C. In another experiment 24 silkworms in the second instar were similarly inoculated and none of them died, the maximum temperature during the latter experiment being 28° C.

PATHOGENESIS

Cutworms inoculated by puncture with pure cultures of *Bacillus noctuarum* become infected and show a mortality of practically 100 per cent. The period from inoculation to the death of the worm varies considerably, depending largely upon the temperature environment. At incubator temperature it may be less than a day, at room temperature it may be two or three days, while during cool weather this period may be even longer. In the few experiments performed with cutworms in which the feeding method of inoculation was used no deaths occurred.

The susceptibility of the larvæ of silkworms (*Bombyx mori* L.) and of hornworms (*Protoparce sexta* Johan. and *P. quinquemaculata* Haw.) to experimental infection with *Bacillus noctuarum* is similar, the mortality being about 100 per cent where the puncture method is employed and much less when feeding inoculations are made. The larvæ of the catalpa moth (*Ceratomia catalpae* Bdv.) and grasshoppers (pl. 2, D) were also tested and found to be readily infected by puncture. No species tested was immune.

Hornworms inoculated in 1921 with a culture [6] of *Bacillus noctuarum* that was isolated in 1917 died in practically the same period as did the worms inoculated with the same culture in 1917. With this culture silkworms were inoculated each year from 1918 to 1921 and all of them died, the period from the inoculation to the death of the worms being about equal in all instances. No important change in virulence, therefore, has yet been observed in *B. noctuarum* kept on artificial media for four years.

Once *Bacillus noctuarum* gains entrance to the blood of the larva it is seen that a fatal outcome under the usual environmental conditions is almost inevitable. That the tissues possess some protective agencies,[7] however, seems probable from observations already made. In microtome sections evidence is gained that in the infected larva a phagocytosis occurs which tends to give some protection to the host. This phenomenon is suggested by the presence of cell groups (Pl. 1, G) in the blood spaces of the sick worms. Some small groups are found in cutworms within one day following a puncture inoculation and after two days the number and size of the groups have increased. The number of groups in a section is never large, only two or three and sometimes none at all being present. Their size varies from 40 to 150 microns in diameter or even more. The smaller groups consist of cells arranged about a single center, while the larger ones may have a conglomerate structure, two or three centers being seen in one section of the group. The cells making up the centers are more or less spherical while the others are somewhat spindle-shaped and arranged in a concentric fashion about a center or group of centers. The same phenomenon is observed in cutworms (Pl. 1, H) and in hornworms (Pl. 1, I) inoculated with *B. sphingidis*. As might be expected in insects as different as are cutworms and hornworms, some differences in the details of the phenomenon are to be found in the two species of worms.

While satisfactory direct ocular proof demonstrating that these cell groups are performing the function of phagocytes is yet wanting, there are certain facts at hand which tend to indicate strongly that they are doing so. These are as follows: (a) Cells with phagocytic power are generally recognized as being present in insects; (b) the cell groups are found in inoculated larvæ and not in uninoculated ones; (c) different investigators have recorded observations to the effect that phagocytosis occurs in bacterial infections; and (d) Speare[8] has shown in fungous infections of cutworms the presence of cell groups which are very similar in structure to those seen in these bacterial infections of this worm, the fungi being easily recognized within the cytoplasm of many of the cells.

A rabbit inoculated intravenously with 1 cubic centimeter of a normal salt suspension from a 24-hour agar culture, containing about 100 million organisms, showed an impaired appetite on the following day, from which it readily recovered. An autopsy on the etherized animal, performed more than a month after inoculation, revealed only a few unimportant lesions.

[6] The culture was on agar kept at room temperature and shielded from the light, transfers being made two or three times a year.

[7] Much indeed is yet to be done on immunity in insects. The problem is receiving some attention at present by different investigators, prominent among whom are Paillot and Metalnikow in France.

[8] SPEARE, A. T. FURTHER STUDIES OF SOROSPORELLA UVELLA, A FUNGOUS PARASITE OF NOCTUID LARVÆ. *In* Jour. Agr. Research, v. 18, p. 417-422. 1920.

COMPARISON OF BACILLUS NOCTUARUM, B. SPHINGIDIS, AND B. ACRIDIORUM

The morphology of *Bacillus noctuarum* (pl. 1, A, B), *B. sphingidis* (pl. 1, C, D), and *B. acridiorum* (pl. 1, E, F) is very similar. Their cultural characteristics, while slightly different (Table I), show that they are closely related species. Cutworms (pl. 2, A, B), grasshoppers (pl. 2, C, D), silkworms (pl. 2, G, I), catalpa moth larvæ (pl. 2, H, J), and hornworms (pl. 2, K, L, M, N) inoculated by puncture with pure cultures of *B. noctuarum* and *B. sphingidis*, respectively, died in each instance from septicemia. Similarly grasshoppers (pl. 2, E, F) and hornworms (pl. 2, O, P) inoculated with *B. acridiorum* died from infection with this species. The virulence of *B. noctuarum* and *B. sphingidis* were almost equal, while that of *B. acridiorum*, at the time of the experiments at least, was less.

The serum of a rabbit immunized with *Bacillus noctuarum* and showing an agglutinin titer of 1:3,200 for the culture used did not agglutinate *B. sphingidis* at any dilution, and the serum of a rabbit immunized with *B. sphingidis* and showing a titer of 1:4,000 for the culture used did not agglutinate *B. noctuarum* at any dilution. Neither of the immune sera would agglutinate the "souche sidi" or "souche cham" strain of *B. acridiorum*.

From the foregoing observations it will be noted that the morphology, cultural characteristics, and pathogenesis of *Bacillus noctuarum*, *B. sphingidis*,[9] and *B. acridiorum* are quite similar, being sufficiently alike to place them in the same group of organisms, an important one consisting of species associated with septicemias encountered among many insects. Serologically, however, the three species are quite different.

It is not unlikely that when further studies have been made on cutworm septicemia other strains of *Bacillus noctuarum* will be encountered which differ from the one described here.

PREDISPOSING CAUSES

The results obtained from the study of the exciting cause of cutworm septicemia recorded above and the knowledge at hand concerning other insect diseases belonging to the same group of disorders lead one to believe that in the causation of the disease the exciting cause receives much aid from predisposing factors. That these contributing causes are important is evident, the problem being one of the interesting ones yet to be solved.

It seems probable that the incidence of the disease in nature varies somewhat with the seasons. High temperature is probably also a contributing agent. Data are yet wanting to show definitely the value of the different instars as predisposing factors. The facts at hand indicate that larvæ in the last stage are more susceptible than they are in any of the other instars. Differences that exist in the susceptibility of the different species and genera of cutworms to infections are likewise not yet established.

[9] In giving two names to two cultures so similar as are *B. noctuarum* and *B. sphingidis* the writer has followed the example of other workers who have encountered and studied different members of the interesting group of bacilli to which these belong. When this group is more completely worked there may develop good reasons for changing the specific classification that is being made.

group of organisms as shown by their

Culture No.	Culture.	Acid formation.																Gas formation.											
		Dextrose.	Galactose.	Levulose.	Mannose.	Saccharose.	Maltose.	Lactose.	Raffinose.	Dextrin.	Inulin.	Erythrite.	Glycerin.	Mannite.	Arabinose.	Xylose.	Salicin.	Plain agar.	Dextrose.	Galactose.	Levulose.	Mannose.	Saccharose.	Maltose.	Lactose.	Raffinose.	Dextrin.	Inulin.	Erythrite.	Glycerin.	Mannite.	Arabinose.	Xylose.	Salicin.	Plain agar.
1	Bacillus sphingidis.....	+	+	+	+	+	+	s	+	+	o	o	+	+	s	+	+	o	s	s	s	s	+	+	+	+	+	o	o	+	+	+	s	o
2	Bacillus from hornworm.	+	+	+	+	+	+	s	+	+	o	o	+	+	+	+	+	o	s	+	s	s	+	+	+	+	+	o	o	+	+	+	s	o
3	Bacillus noctuarum	+	+	+	+	+	+	s	+	+	o	o	+	+	+	+	+	o	s	+	s	s	+	+	+	+	+	o	o	+	+	+	s	o
4	Bacillus from cutworm.	+	+	+	+	+	+	s	+	+	o	o	+	+	+	+	+	o	s	+	s	s	+	+	+	+	+	o	o	+	+	+	s	o
5	Bacillus acridiorum (strain souche sidi.).	+	+	+	+	+	+	s	+	+	o	o	+	+	+	+	+	o	s	+	s	s	+	+	+	+	+	o	o	+	+	+	s	o
6	Bacillus acridiorum (strain souche cham).	+	+	+	+	+	+	s	+	+	o	o	+	+	+	+	+	o	s	+	s	s	+	+	+	+	+	o	o	+	+	+	s	o

[a] Agar containing 1 per cent of carbohydrate and Andrade's indicator, were used in making the determinations recorded in Table I. The presence of a decided amount of acid or gas is indicated by +, and their absence by o. A slight amount of either is indicated by s. Where only a slight amount of acid was produced the reaction soon changed to alkaline. Where a small quantity of visible gas is present, shown from the presence of a bubble or two within the differential agar, it was learned that even this amount was not encountered in all of such tubes inoculated. In the identification of a species a slight variation in the cultural characters as given in the table may be expected and allowed.

Culture No. 1, *B. sphingidis*, was isolated from a dead hornworm in September, 1917, at Clarksville, Tenn. No. 2 was isolated, July, 1921, from a dead hornworm sent by A. C. Morgan from Clarksville. It is *B. sphingidis* and probably the same strain as in No. 1; its immunologic relation to No. 1, however, was not determined. No. 3, *B. noctuarum*, was isolated from a dead cutworm September, 1917, at Clarksville, Tenn. No. 4 was isolated from each of two dead cutworms, received June, 1921, from Dr. F. H. Chittenden, of Truck Crop Insect Investigations, Bureau of Entomology, the worms having been collected from Bathurst, N. B., by S. B. Bond. Cultures Nos. 5 and 6 are *B. acridiorum*, strain souche sidi and souche cham, respectively, received from Bathurst, N. B., by S. B. Bond, September, 1921.

It will be observed that the cultures in the table fall into two subgroups, one in which very little visible gas is produced, represented by the first three cultures; and a second one, which includes the last three cultures, in which much gas is formed. The members of the first subgroup are very similar throughout. Culture No. 4 from cutworm is quite similar to *B. acridiorum*, strain "souche cham," differing chiefly in lactose. *B. acridiorum* "souche sidi" and *B. acridiorum* "souche cham" differ chiefly in the gas formation in lactose and glycerin.

SYMPTOMS AND POST-MORTEM CHANGES

Worms infected by puncture become sluggish and cease to feed. The feces become thin and are slowly discharged, and a watery fluid oozes from the mouth. The turgidity and plumpness which characterize the appearance of the healthy larvæ are lost in those that are sick. Death occurs in from two to four days as a rule after puncture inoculation, the period depending largely upon the temperature environment of the worms.

After death the remains of the worms are soft, and the color changes to a brown which deepens as the process of decay continues. During the decomposition of the tissues a thick, brown, nonviscid mass is formed which on drying becomes brittle. The chitinous wall continues intact and on drying the remains become a shriveled, more or less black, mummified mass that retains in general the original form of the worm. The decay is accompanied by a slight odor, which is at no time disagreeable.

It seems probable that there might be disturbances within the alimentary tract from the ingestion of *Bacillus noctuarum* without resulting in a septicemia. The symptoms of such a condition, if indeed it occurs at all, are yet to be learned.

MODE OF TRANSMISSION

The portal of entry of the infecting organism in cutworm septicemia as pointed out above, has not been definitely determined. It has been demonstrated, however, that in the diseased worm the causal bacillus multiplies rapidly in the blood and within the alimentary tract, furnishing thus a source for an increase of the germ. In moist soil the bacillus remains viable over long periods, continuing in this way the possibility for infection.

From the observations yet recorded it does not seem that this disease in nature spreads readily, at least during the more active growing season for the crops on which cutworms feed. One is led to expect that such might be true from the observation that the infection is not easily transmitted experimentally through feeding inoculations. These facts, together with the observation that *Bacillus noctuarum* is readily destroyed through drying, point to the conclusion that the use of cultures of this organism can not be recommended at the present time as an economic measure for the artificial control of the disease.

DIAGNOSIS, PROGNOSIS, AND TREATMENT

If cutworms seem sluggish, cease to feed, and die, and the remains become soft and turn brown to almost black, cutworm septicemia may be suspected. The disease may be more strongly suspected if healthy worms inoculated with material from dead ones show symptoms and post-mortem changes which have been noted for the disorder. To make a positive diagnosis, however, it is necessary to demonstrate the presence of *Bacillus noctuarum* in the sick larvæ or the remains of those recently dead. Microscopic preparations made from worms sick or recently dead of the disease will contain numerous, short, nonspore-bearing rods. Agar plates streaked with the tissues of such worms will show in 24 hours at room temperature a well-defined bluish gray growth of an actively motile bacillus.

The prognosis in an infected worm in which a septicemia has actually occurred is particularly grave. Cases in which the infecting organism can be demonstrated in the blood probably all die, especially if the temperature at which the worms are kept is such as ordinarily prevail

uring the warmer half of the year. Cases, if there are such, which suffer om cutworm septicemia but in which no actual septicemia has occurred, would seem, must have a particularly favorable prognosis. One is d to this belief from the low mortality that follows feeding inoculations. Those who rear cutworms for purposes of study and who make observaons on them over a considerable period, are interested in avoiding losses om cutworm septicemia. To them preventive measures are suggested s the treatment which offers the greatest promise. Sterilization of the oil and cages used could easily be accomplished by steaming, since *acillus noctuarum* is readily destroyed by heat. Other facts given in the resent paper will aid in devising efficient means for reducing losses from is disorder.

SUMMARY

Entomologists have observed that sometimes cutworms die and the emains soften and turn brown, which deepens into almost black.

The results of a study of this condition show that it is an infectious lisease in which there is a marked septicemia preceding death.

The name cutworm septicemia is here suggested and used for the disrder.

This infection produced by puncture inoculation runs a course of from wo to four days, the period depending very much upon the temperature.

The most prominent symptoms of the experimentally produced disease re a lessened appetite and finally its failure, listlessness, a lack of turgidity of the body, a diarrhea, a thin discharge from the mouth, and death.

The bacterial species occurring in the septicemia is demonstrated o be a short, actively motile bacillus to which the name *Bacillus noctuarum* is here given and used.

Bacillus noctuarum remains alive for a long period in a moist environment at ordinary temperature but is readily destroyed by heat and by drying, being quite susceptible to direct sunlight and to chemical disinfectants.

The septicemia is not readily produced by feeding but is readily produced by puncture inoculations, the mortality then being approximately oo per cent.

Hornworms, silkworms, catalpa-moth larvæ, and grasshoppers are lso susceptible to inoculation with *Bacillus noctuarum* when the punc- ure method is employed.

The change, if any, in the virulence of a culture of this bacillus after our years on artificial media has been slight.

Bacillus noctuarum is similar in many respects to *B.* (*Coccobacillus*) *cridiorum* and to *B. sphingidis*. Serologically they are distinctly ifferent.

Probably the disease is transmitted in nature most often by way of the limentary canal.

Cutworm septicemia may be suspected from the symptoms and postnortem changes. The diagnosis is definitely made by finding *Bacillus octuarum* present in large numbers.

Apparently a comparatively small percentage of cutworms die of this lisease in the field during the more active growing season of the crops on which they feed.

Preventive treatment is suggested to those making studies on cutworms nd wishing to reduce the loss of insects due to this infection.

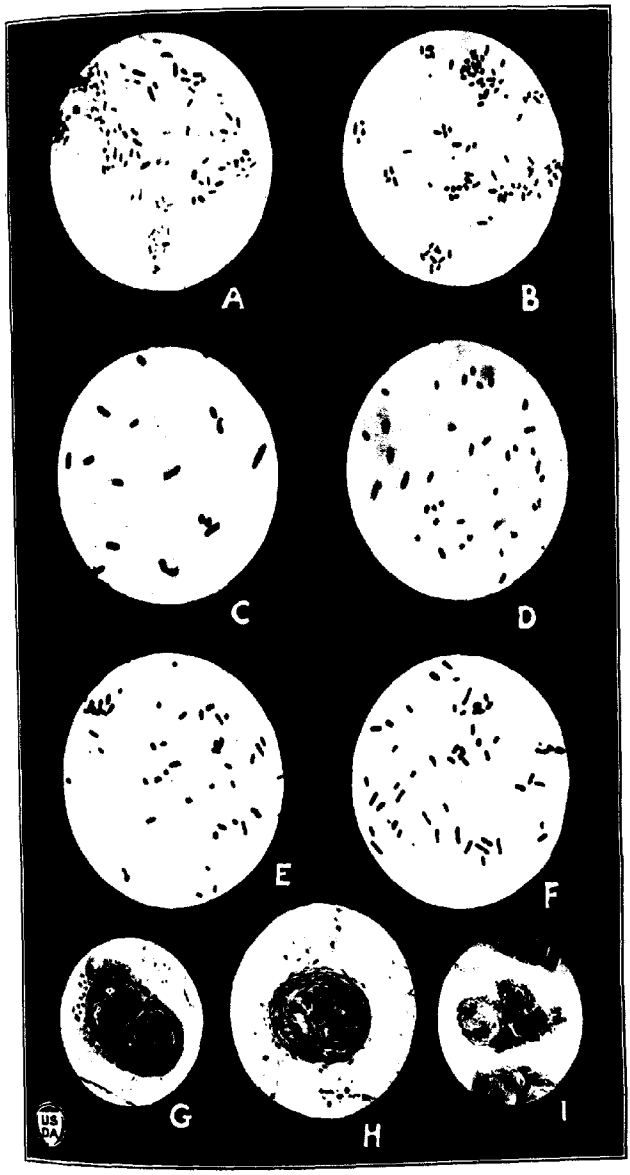
While there is much yet to be learned about cutworm septicemia the acts already determined and given in the present paper will suffice to nswer many questions likely to arise in connection with this disease.

PLATE 1

Cutworm septicemia

Photomicrographs of bacilli from 1-day agar cultures and from 4-day bouillon cults. All magnified 1,500 diameters. Also photomicrographs of cell groups in blood spaces suggesting phagocytosis. These are magnified 100 diameters.

A.—*Bacillus noctuarum* from bouillon.
B.—*Bacillus noctuarum* from agar.
C.—*Bacillus sphingidis* from bouillon.
D.—*Bacillus sphingidis* from agar.
E.—*Bacillus acridiorum* from bouillon, strain "souche sidi."
F.—*Bacillus acridiorum* from bouillon, strain "souche cham."
G.—Cell group in cutworm inoculated 2 days with *Bacillus noctuarum*.
H.—Cell group in cutworm inoculated 2 days with *Bacillus sphingidis*.
I.—Cell group in hornworm inoculated 1 day with *Bacillus sphingidis*.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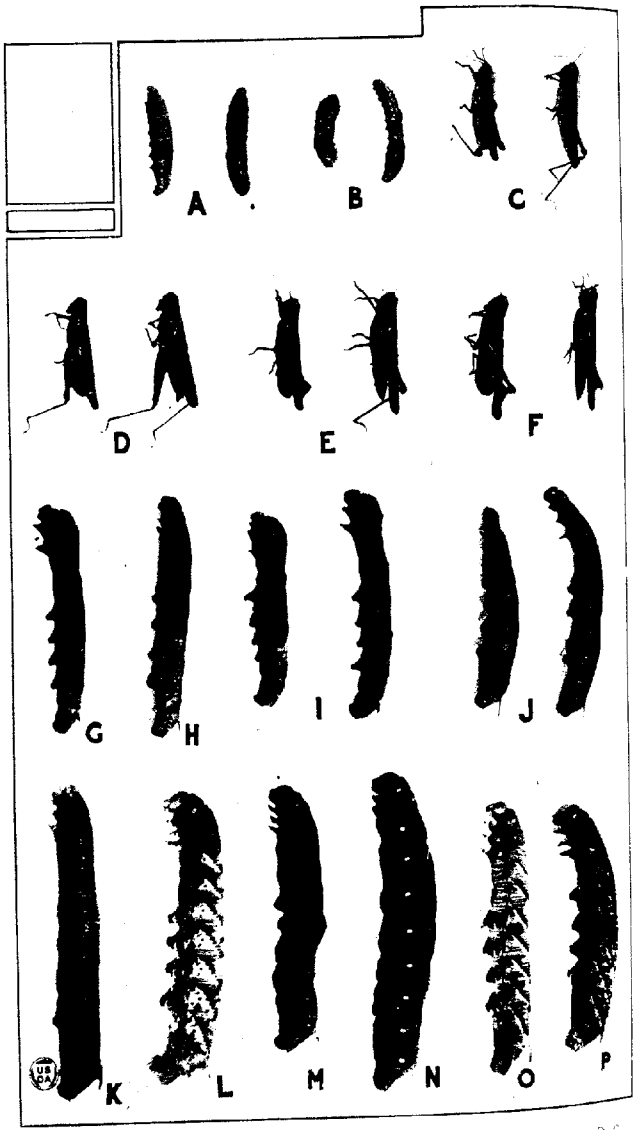
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PLATE 2

Cutworm septicemia

Insects dead from inoculation with pure cultures by the puncture method, reproduced at approximately two-thirds natural size.

A.—Cutworms inoculated with *Bacillus noctuarum*.
B.—Cutworms inoculated with *Bacillus sphingidis*.
C.—Grasshoppers inoculated with *Bacillus sphingidis*.
D.—Grasshoppers inoculated with *Bacillus noctuarum*.
E.—Grasshoppers inoculated with *Bacillus acridiorum*, strain "souche sidi."
F.—Grasshoppers inoculated with *Bacillus acridiorum*, strain "souche cham."
G.—Silkworm inoculated with *Bacillus sphingidis*.
H.—Catalpa moth larva inoculated with *Bacillus sphingidis*.
I.—Silkworms inoculated with *Bacillus noctuarum*.
J.—Catalpa moth larvæ inoculated with *Bacillus noctuarum*.
K.—Hornworm inoculated with *Bacillus sphingidis* (Culture 1 of Table I).
L.—Hornworm [1] inoculated with *Bacillus sphingidis* (Culture 2 of Table I).
M.—Hornworm inoculated with *Bacillus noctuarum*.
N.—Hornworm inoculated with bacillus from cutworm (Culture 4 of Table I).
O.—Hornworm [1] inoculated with *Bacillus acridiorum* ("souche sidi").
P.—Hornworm inoculated with *Bacillus acridiorum* ("souche cham").

[1] The dark points on the lateral surface of the larvæ are from parasitism with *Apanteles congregatus*.

A STUDY OF THE SEROLOGY, THE CEREBROSPINAL FLUID, AND THE PATHOLOGICAL CHANGES IN THE SPINAL CORD IN DOURINE[1]

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HISTORY OF THE CASE

The subject supplying the material for this study was a 15-year-old brown stallion, No. 128, which had contracted dourine under natural conditions. The serum of this animal gave a positive reaction to the complement-fixation test for dourine in 1913 in the course of routine diagnosis in connection with the campaign of control and eradication of dourine conducted by the Bureau of Animal Industry and the various States infected. This animal was purchased from its owner in Montana and shipped to the Bureau Experiment Station at Bethesda, Md., in 1913, together with 16 other horses giving positive reactions to the complement-fixation test for dourine, for observation and study. Dourine was found to be quite prevalent in the section of the State from which this animal was obtained. The animal at the time of purchase was 7 years old, in excellent condition, and showed no clinical evidence of dourine. Repeated examinations of the blood for the presence of trypanosomes were negative.

This stallion was bred a number of times to a native mare (i. e., an eastern mare), No. 103, free of dourine infection, to determine whether the infection could be transmitted by him. The first service was November 10, 1913. On September 19, 1914, the mare aborted. She had been losing flesh gradually and exhibiting evidence of muscular weakness, but gave no other indication of dourine infection. On September 24 the mare died. Serum collected before death, however, gave a negative reaction to the complement-fixation test for dourine. The stallion was kept under continued observation, but at no time did he show any clinical evidence of dourine. He developed into a good work animal and was used for this purpose for several years.

During the two years 1920 and 1921 the animal was not worked and gradually fell away in flesh. During 1921 symptoms indicating an affection of the central nervous system appeared from time to time. The animal would turn rapidly in a circle in one direction for several minutes, sometimes falling to the ground, and after several minutes he would rise and be apparently normal. He was found down December 10, 1921, and dead the next morning.

POST-MORTEM FINDINGS

On post-mortem examination a gelatinous infiltration was noted in the subcutaneous tissue and the abdominal muscles. The penis was normal. The left testicle was atrophied, being about half the normal size. The glandular substance was soft and flabby and was tightly

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adherent to the testicular coverings and could be stripped out only with great difficulty. The right testicle was normal. The thoracic organs and the liver, kidney, and intestines were normal. The spleen was slightly thickened and showed areas of hemorrhagic infarction. There was a gelatinous infiltration in the dorsal and lumbar portion of the spinal canal.

COMPLEMENT FIXATION TESTS OF SERUM

Samples of serum were drawn from this animal from time to time between the years 1913 and 1921 and were subjected to the complement-fixation test for dourine. Quantitative tests of the serum were made, starting with 0.2 cc. and decreasing to 0.03 cc.

TECHNIC OF THE TEST.

The hemolytic system consists of 1 cc. of a 3 per cent suspension of washed sheep corpuscles, 2½ units of hemolytic amboceptor, and 1½ units of complement, the latter being titrated each day a test is made. The antigen consists of a suspension of *Trypanosoma equiperdum*, the causative agent of the disease, and is recovered from the blood of artificially infected rats, as described in a previous paper.[1] Two to three units of antigen are used in the test, provided four times this amount shows no anticomplementary action. The serum is inactivated at 58° C. for 35 minutes in physiological salt solution.

TABLE I.—*Samples of blood serum of horse 128 tested at intervals during 1913–1921*

Date.	Varying quantities of serum (cubic centimeters).										Serum control (cubic centimeters)
	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1	0.15	0.2	0.3
Nov. 15, 1913	.....	[a] Sl.	Sl.	[b] 1+	1+	[c] 2+	2+	2+	[d] 4+	4+	–
Dec. 23, 1913	.....	–	Sl.	Sl.	1+	2+	2+	[d] 3+	4+	4+	–
Mar. 6, 1914	.....	Sl.	Sl.	Sl.	1+	1+	1+	2+	3+	4+	–
May 22, 1914	.....	Sl.	Sl.	1+	1+	1+	2+	2+	3+	4+	–
Aug. 13, 1914	Sl.	2+	3+	4+	4+	4+	4+	4+	4+	4+	–
Feb. 28, 1916	–	–	–	–	1+	3+	3+	3+	3+	4+	–
Jan. 11, 1917	–	–	Sl.	Sl.	1+	1+	2+	4+	4+	4+	–
Dec. 14, 1917	–	Sl.	2+	2+	3+	4+	4+	4+	4+	4+	–
Mar. 14, 1919	–	–	–	Sl.	Sl.	1+	1+	2+	2+	3+	–
Mar. 19, 1920	–	Sl.	Sl.	Sl.	Sl.	Sl.	1+	2+	3+	3+	–
Nov. 2, 1921	–	–	–	–	1+	1+	1+	2+	2+	3+	–

[a] Sl.=less than 25 per cent fixation of complement.
[b] 1+=25 per cent fixation of complement.
[c] 2+=50 per cent fixation of complement.
[d] 3+=75 per cent fixation of complement.
[e] 4+=100 per cent, or complete fixation of complement.

As will be seen from Table I, the serum of horse 128 at no time gave a 4+ reaction with a quantity lower than 0.06 cc., and with this amount only on one occasion. The serum titrations in general show a fairly constant result with several exceptions. Whether these exceptions (August 13, 1914, and December 14, 1917) are indications of a fluctu-

[1] REYNOLDS, F. H., and SCHOENING, H. W. AN IMPROVED METHOD FOR RECOVERING TRYPANOSOMES FROM THE BLOOD OF RATS FOR ANTIGEN PURPOSES IN CONNECTION WITH COMPLEMENT FIXATION. In Jour. Agr. Research, v. 14, p. 573–576. 1918.

ating antibody content or are a result of a more sensitive antigen and closer hemolytic system on these test dates is problematical. The average of these titrations shows this serum to be only a mildly positive one. Very frequently we have encountered serums from cases of natural infection which gave a 4 + reaction with 0.005 cc. or less. The titrations of the serum from 1919 show a decrease in antibodies, so that a 4+ reaction is not obtained even in quantities of 0.2 cc.

EXAMINATION OF SPINAL FLUID

Previous to the post-mortem examination spinal fluid was drawn from the axis-atlas articulation by means of a sterile trocar into sterile tubes. A good specimen of fluid was obtained free of any red cells or other contamination. It was immediately taken to the laboratory, where it was subjected to the colloidal gold test, the globulin test, and a cell count, as well as a complement-fixation test for dourine.

THE COLLOIDAL GOLD TEST

The extensive application of the colloidal gold test, since its inception by Lange[3] in 1912, to cerebrospinal fluids of patients affected with syphilis in which the central nervous system was involved has established for it a place as one of the tests indicated in the routine diagnostic work on this disease.

A preliminary report on the application of the colloidal gold test to spinal fluids of horses affected with dourine was made by one of the writers as a co-author with Reynolds.[4] In that work spinal fluids from horses whose serums gave positive reactions to the complement-fixation test for dourine were subjected to the colloidal gold test. The spinal fluids were obtained from the horses immediately subsequent to their deliberate destruction, which was done in the course of the campaign for the control and eradication of dourine. As was expected, various reactions were obtained with these fluids, as the animals destroyed were in various stages of the disease. However, a number of reactions were obtained which bore considerable similarity, but no interpretation could be placed on them, as the spinal cords were not available for histopathological study.

The test involves the precipitation of colloidal gold by spinal fluid altered as a result of disease. The technic of the test is comparatively simple. The greatest difficulty is encountered in the preparation of a satisfactory solution of colloidal gold. The method of Miller, Brush, Hammers, and Felton, etc.,[5] was used in the preparation of the gold solution, and as a rule a satisfactory solution was prepared.

The technic of the test consists in setting up a rack with 11 tubes, in the first of which is placed 1.8 cc. of a 0.4 per cent sodium chlorid solution and in the remaining 10 tubes 1 cc. of the same solution. In the first tube is placed 0.2 cc. of the spinal fluid, making a dilution of 1 to 10. After thoroughly mixing, 1.0 cc. from this tube is placed in the second tube,

[3] LANGE, Carl. UEBER DIE AUSFLOCKUNG VON GOLDSOL DURCH LIQUOR CEREBROSPINALIS. *In* Berlin Klin. Wchnschr., Jahrg. 49, p. 897-901, 5 fig. 1912.

[4] REYNOLDS, Francois H. K., and SCHOENING, Harry W. THE PRECIPITATION OF COLLOIDAL GOLD IN THE CEREBROSPINAL FLUID OF HORSES WITH DOURINE. *In* Jour. Infect. Diseases, v. 31, p. 59-63. 1922.

[5] MILLER, SYDNEY R., and others. A FURTHER STUDY OF THE DIAGNOSTIC VALUE OF THE COLLOIDAL GOLD REACTION, TOGETHER WITH A METHOD FOR THE PREPARATION OF THE REAGENT. *In* Bul. Johns Hopkins Hosp., v. 26, p. 392-407, 3 charts, pl. 30-31. 1915. References, p. 407.

and 1 cc. from the second tube is transferred to the third tube, and so on until the tenth tube, 1 cc. from this tube being discarded. This procedure gives a dilution of the spinal fluid of from 1 to 10 to 1 to 5,120, the eleventh tube being a control on the gold solution. Five cubic centimeters of the colloidal gold solution is then added to each tube, the rack shaken and left at room temperature, and the reading made in 24 hours. The reading is made according to the amount of precipitation which takes place in each tube and is recorded on a form shown in Figures 1 to 4. Five on the scale, or colorless, represents complete precipitation; 4, 3, 2, and 1 are varying degrees of precipitation, and 0, or red, indicating no change of the solution.

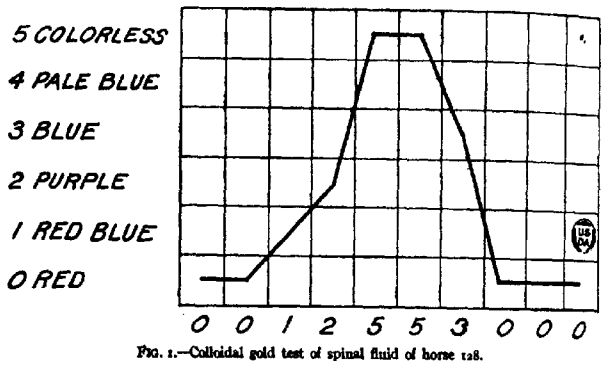

FIG. 1.—Colloidal gold test of spinal fluid of horse 128.

The spinal fluid from horse 128 gave a reaction to the colloidal gold test as shown in Figure 1.

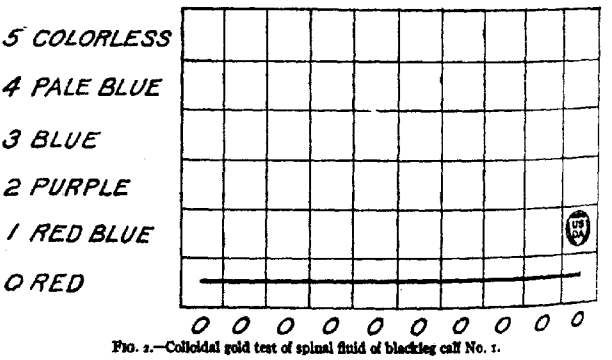

FIG. 2.—Colloidal gold test of spinal fluid of blackleg calf No. 1.

Spinal fluids from three calves, No. 1, 2, and 3, dead of artificial blackleg infection, were used as negative controls. The testing of a number of spinal fluids from such blackleg infected animals usually results in 10 naughts. In a few cases, however, a No. 1 change on the scale in several of the tubes was noted. The results are shown in figures 2, 3, and 4. No spinal fluid from a normal equine was available for control purposes.

TEST FOR GLOBULIN

The Ross-Jones [6] test for globulin was applied to the spinal fluids of horse 128 and blackleg calves 1, 2, and 3. The test consists in layering cc. of spinal fluid on 2.0 cc. of a saturated solution of ammonium sulphate. A white or gray ring at the point of contact of the two fluids indicates a positive reaction. The spinal fluid from horse 128 gave a

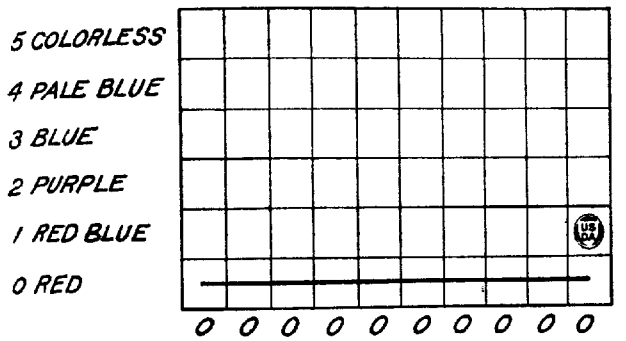

FIG. 3.—Colloidal gold test of spinal fluid of blackleg calf No. 2.

markedly positive reaction, while the fluid from calves 1, 2, and 3 gave a clear-cut negative reaction.

CELL COUNT

For the cell count the ordinary white corpuscle pipette and blood-counting chamber were used. The diluting fluid consisted of 0.3 per

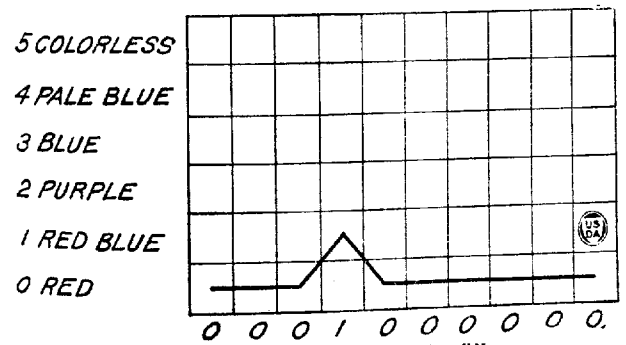

FIG. 4.—Colloidal gold test of spinal fluid of blackleg calf No. 3.

cent glacial acetic acid in distilled water. An average of three counts of fluid from horse 128 showed 180 cells per cubic millimeter. In the three fluids from the blackleg calves no cells were observed. As the cells in normal fluids may vary from 0 to 10, the count of 180 in the fluid of

[6] ROSS, GEORGE W., and JONES, ERNEST. ON THE USE OF CERTAIN NEW CHEMICAL TESTS IN THE DIAGNOSIS OF GENERAL PARALYSIS AND TABES. *In* Brit. Med. Jour., 1909, v. 1, p. 1111-1113. 1909.

horse 128 is significant in that this is one of the important indications of alteration in the central nervous system.

COMPLEMENT-FIXATION TEST

The spinal fluids of horse 128 and blackleg calves 1, 2, and 3 were subjected to the complement-fixation test for dourine. The spinal fluids were not inactivated. Fluid from horse 128 gave a 4+ reaction in a quantity as low as 0.05 cc., 0.2 cc. in the control tube showing no inhibition of hemolysis. Fluids from blackleg calves 1, 2, and 3 gave negative results to the test. Data of the tests are given in Table II.

It is of interest to note that the spinal fluid of horse 128 gave a 4+ reaction with 0.05 cc., while the serum of this animal tested at the same time against the same antigen and hemolytic system gave only a 3+ reaction with 0.2 cc.

TABLE II—*Complement-Fixation Tests of Spinal Fluids*

Animal.	Quantity of fluid (cubic centimeters).												Control (cubic centimeters).	
	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.1	0.15	0.2	0.5	0.2	0.5
Horse No. 128	a−	b1+	c2+	d3+	e4+	4+	4+	4+	4+	4+	4+	.....	−	.....
Blackleg calf No. 1	.....	.....	.....	.....	.....	.....	.....	.....	−	.....	−	−	.....	−
Blackleg calf No. 2	.....	.....	.....	.....	.....	.....	.....	.....	−	.....	−	−	.....	−
Blackleg calf No. 3	.....	.....	.....	.....	.....	.....	.....	.....	−	.....	−	−	.....	−

a −=Complete hemolysis.
b 1+=25% fixation of complement.
c 2+=50% fixation of complement.
d 3+=75% fixation of complement.
e 4+=100% fixation of complement.

MICROSCOPIC FINDINGS

The microscopic examination of tissues from this case is intended merely to supplement the spinal fluid studies. No attempt has been made to study the changes in the peripheral nerves, the larger nerve trunks, the sympathetic system, or the spinal ganglia. The spinal cord alone was used. Sections were made from the dorsal and lumbar regions of the cord, the principal changes described herein being found in those from the lumbar region.

It was first necessary to ascertain if any changes could be seen by the ordinary routine method of staining with hematoxylin and eosin. This was followed by the more specialized stain, Pal's modification of the Weigert method, and lastly by Heller's myelin-sheath stain, which by virtue of the osmic acid is more sensitive to degenerative changes than the universally used Weigert method or its modifications.

The microscopic changes were not so pronounced as might have been expected from the long duration and chronic character of the case. The dura mater was thicker than usual, but no excessive hypertrophy could be seen in the fibrous tissue or in the number of fixed connective-tissue cells. The fibrous bundles were apparently somewhat thicker than normal. There was a slight thickening of the walls of the blood vessels, which were well filled but not overdistended. No hemorrhages

were present in any portion of the dura mater. The pia mater showed no appreciable changes.

In the nerve tissues proper the alterations will be considered under three headings—vascular changes, neuroglia changes, and degenerative changes—which may affect the entire neuron comprising both the nerve cell and the nerve fiber, or more often only the nerve fiber. For the sake of convenience the changes observed in the nerve cells and those noted in the nerve fibers will be described separately.

VASCULAR CHANGES

Section stained with hematoxylin and eosin showed good contrast between the gray and white substance. A number of well-distended capillaries were noted in different parts of the dorsal column and at the point of entrance of the sensory fibers. In the lateral and ventral columns the distention of the capillaries was less pronounced except those entering the ventral median fissure. In the lateral horns of the gray substance near the outer border the capillary distention was quite marked, suggestive of hemorrhages.

NEUROGLIAR CHANGES

While neurogliar changes are not so appreciable with the hematoxylin and eosin stain as with the more delicate silver impregnation of the Golgi method or the gold-impregnation method, which bring out besides the neuroglia also the spider cells, nevertheless an increase in the amount of neuroglia can be observed both in the white substance and in the gray substance. This increase is less in the ventral columns than in the lateral and dorsal columns. The increase of neuroglia on either side of the dorsal septum is quite perceptible, verging on sclerosis, and to a less degree at the dorsolateral groove and the lateral columns, while in the dorsal columns it is in excess of that in the ventral columns. In the gray substance the neurogliar increase is seen in the gray commissure around the central canal and in the central gelatinous substance, as well as in the ventral and dorsal horns, especially in the Rolandic substance capping the dorsal horns. The central canal was open but not distended. The single row of ependima cells appeared unaltered.

The ganglion cells stained with hematoxylin and eosin showed the neuroplasm, nucleus, and in some of the cells the nucleolus of the motor cells unaltered. The sensory cells and the cells in the column of Clark were smaller in size, which might have been due to the presence of lymph contained in the perceptibly distended pericellular lymph spaces surrounding the sensory ganglion cells. This, however, is somewhat questionable, as the increased amount of lymph in the pericellular lymph spaces did not cause any appreciable cytologic changes in either the motor or the sensory ganglion cells.

DEGENERATIVE CHANGES

Degenerative changes in the myelin of the medullated nerve fibers could not be detected by the hematoxylin and eosin stain. Pal's modification of the Weigert method, while not productive of conclusive results, gave some indications of beginning degenerative changes, which were manifested by the lighter color effect in the degenerated fibers as

contrasted with the darker stained normal fibers. In the dorsal columns slight change could be observed in the outer portion of Burdach's columns near the periphery and close to the dorsolateral groove. No changes were noted in the columns of Gall. The yellowish tint in the medullated fibers extended into the lateral columns, gradually fading out, and entirely disappearing in the ventral columns. After staining with osmic acid according to Heller's method, the degenerative changes, as indicated by the brownish black deposits or clumps, were more in evidence. A large number of black clumps were present at the dorsolateral groove, the point of entrance of the extra medullary fibers constituting the dorsal roots. The clumps gradually decreased in number as the fibers entered the gray substance of the dorsal horn, and almost entirely disappeared in the ventral horns.

The degeneration of the medullated fibers is quite as apparent and may bear some significance to the clinical symptoms. The largest number of black clumps were found in the medullary fibers of the outer dorsal column known as Burdach's column, or funiculus cuneatus. The distribution of the black clumps was not uniform, but varied as to outer and inner, external or internal, situation of the fibers. There was also a difference in the size of the clumps, the larger ones suggesting more complete, and the smaller ones less complete, degenerative changes. The largest number of black clumps were present in the fibers nearest to the dorsal roots or in the outer and external portion of Burdach's columns. The number of clumps diminished in the direction of the dorsomedian septum and also in the direction of the gray commissure. In the inner portion of the dorsal column known as Gall's column, or funiculus gracilis, fewer black clumps were present than in Burdach's column, and they almost entirely disappeared in the fibers nearest to the dorsomedian septum and in the fibers in the region of the gray commissure. The clumps varied in sizes, a few of the larger ones being intermingled with the scattered smaller clumps.

It can be seen from the above described distribution of the black clumps that the degeneration in the medullated fibers was present a greater degree in the dorsal columns, to a less extent in the fibers that portion of the lateral columns nearest to the dorsal roots, and scarcely affected the fibers nearest to the ventral roots and the fibers the ventral columns. In other words, the degenerative changes were confined largely to the dorsal and lateral tracts of the cord.

SUMMARY

A study of the serology, the cerebrospinal fluid, and the pathologic changes in the spinal cord of a stallion dead of dourine infection contracted naturally is reported. This animal was under observation from 1913 to 1921. The serological study covers a period of eight years samples of blood serum being drawn at intervals and subjected to the complement-fixation test for dourine.

The spinal fluid of this animal was subjected to the colloidal gold test a test for globulin and a cell count. The spinal fluid was also subjected to a complement-fixation test for dourine, a significant feature of which was the fact that complete fixation of complement was obtained with 0.05 cc. of spinal fluid, whereas complete fixation of complement was not obtained with 0.2 cc. of blood serum.

PATHOLOGICAL CHANGES IN THE SPINAL CORD

Slight hypertrophy and pronounced capillary distension were present
n the dura and pia mater, but no visible alteration in the arachnoid.
he capillary fullness in the lateral horns of the gray substance suggestive
f hemorrhage was less pronounced in the white substance.

Neurogliar changes were present in the gray and white substance, but
ere better seen in the lateral columns when stained by the Golgi method.

The sensory ganglion cells were somewhat shrunken, while the motor
lls were practically unaltered in outline.

Degenerative changes in the myelin were quite visible by the Pal-
eigert hematoxylin method, and even more so by Heller's osmic-acid
ethod. It is more readily seen in the fibers of the dorsal than the
teral column and is scarcely found in the ventral column.

A BUDROT OF THE PEACH CAUSED BY A SPECIES OF FUSARIUM[1]

By JOHN W. ROBERTS

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In July 1920, peach twigs having numerous dead and blackened buds were sent me from Georgia by Leslie Pierce, of the Office of Fruit Disease Investigations, Bureau of Plant Industry. It was stated that the tree from which the collections were made was a fairly well grown specimen of the variety "Queen of Dixie" peach and that dead buds were present in considerable numbers. The injury was not entirely confined to the buds, there being also a slight discoloration of the twig near the axils. Judging from the size and state of development of the buds, death occurred early in the spring, at a time when they had just begun to swell. At first it was thought that Monilia might be responsible for the injury, but no conidia of Monilia could be found on the buds as received or after keeping them in a moist chamber for 48 hours. However, conidia of a species of Fusarium were found to be present under both conditions. After the 48 hours in a moist chamber, they were particularly abundant, being produced in white sporodochia, dotting the surfaces of the bud scales. The same fungus was found in diseased buds of the variety "Queen of Dixie" peach, also sent in from Georgia during the early spring of 1922 by John C. Dunegan of the Office of Fruit Disease Investigations, Bureau of Plant Industry.

Aderhold[2] described a budrot of the sour cherry which he showed was caused by *Fusarium gemmiperda* sp. n. Buds killed by this disease did not remain on the trees throughout the summer, as is usually the case when attacked by Monilia, but by the development of an abscission layer were made to fall early in the season. No injury to the trees themselves was observed by Aderhold, but the crop of fruit was much reduced. The principal points in Aderhold's description of the fungus are as follows: Dead buds placed in moist chambers developed snow-white sporodochia in five to six days. The conidia were at first nonseptate, most of them later becoming triseptate, somewhat curved, at first barrel-shaped or cylindrical, later sickle-shaped, pointed at both ends, contents hyaline or somewhat granular, later a large vacuole in each cell. They were variable in size, according to age, usually being between 35–45 × 4–5.5 microns. Individually the conidia were colorless, but with age they took on a reddish color in mass, especially beautiful in artificial culture. The conidiophores were either long or short and arose from neighboring branches in large numbers. There were no "Köpfchen" which are often found on aerial conidiophores of species of Fusarium, and no chlamydospores. Mycelial concretions, consisting of cartilaginous dirty white or yellowish masses of hyphae, were present. They were thought to be the beginnings of sclerotia, but no further development took place. Even

[1] Accepted for publication Nov. 1, 1923.

[2] ADERHOLD, Rudolf. EIN DER MONILIAKRANKHEIT ÄHNLICHER KRANKHEITSFALL AN EINEM SAUERKIRSCHBAUME. *In* Zeitschr. Pflanzenkrank., Bd. 11, p. 65–73, pl. 2. 1901.

after three months they were only tangled masses of hyphae, and after five months they had formed neither sclerotia nor fruiting bodies.

In cultures on gelatin and on bread, beads of water appeared. Growth was cottony, snow-white at first, later peach-bloom red, changing to yellow, which finally disappeared. Conidia and mycelial concretions were formed.

The species isolated from buds of the Georgia peach resembles very closely *Fusarium gemmiperda* Aderhold and will be considered as identical with it. This species on rare occasions produces chlamydospores (fig. 1, C), but the writer grew the fungus on artificial media for two years before he found any of them. The peach-bloom red is not so evident as with Aderhold's fungus. The extreme length of the conidia is greater, but if one considers only triseptate conidia, since they predominated in Aderhold's cultures, then the measurements of the two correspond very closely. The form from Georgia peach buds has conidia typically sickle shaped (pl. 1, B), sometimes distinctly broader in the upper third, rather suddenly constricted at the apex and often so at the base, 3 septate or 5 septate forms predominating (fig. 1, A and B). Aerial hyphae may be present, 2 to 8 mm. high, or nearly absent; when absent, pseudopionnotes are abundant; when present, sporodochia are usually formed. Chlamydospores are rarely found. Color of aerial mycelium is white; color of pseudopionnotes is pale pink to cream changing to brown when old. Sporodochia (pl. 1, A) on peach bud scales are white, in cultures from white to salmon pink. Conidiophores may be nearly lacking, being scarcely more than swollen places along the mycelium or they may consist of numerous branches coming out at adjacent points along a hyphal filament. Cream-colored sclerotia may be present, especially on potato plugs.

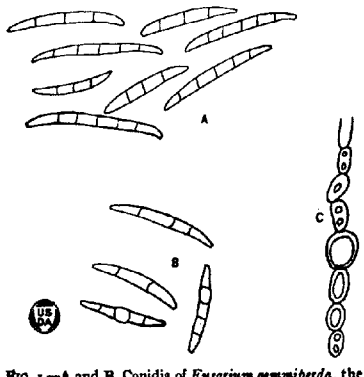


FIG. 1.—A and B, Conidia of *Fusarium gemmiperda*, the latter from old cultures. C, Chlamydospores.

On bud scales of peach 3 to 5 septate conidia were found in large numbers; sometimes the former, but more often the latter, predominating. On cornmeal agar sometimes 3 and sometimes 5 septate conidia predominate regardless of age. On beef agar, potato agar, and potato plugs, 3 septate conidia are usually present in much larger numbers than 5 septate conidia. On 4 per cent potato agar often all are 3 septate.

Conidial measurements are as follows:

Conidia from 30 days' old corn-meal agar:
- 3 septate, 32–46 x 4–5, average 36 x 4.5.
- 4 septate, 42–53 x 4.5, average 47 x 4.5.
- 5 septate, 46–61 x 4.5, average 51 x 4.5.

Conidia from 20 days' old potato plug:
- 2 septate (only 1 measured) 25 x 5, average 25 x 5.
- 3 septate, 29–46 x 4–5, average 38 x 5.
- 4 septate, 38–59 x 4–5, average 46 x 5.
- 5 septate, 42–63 x 4–5, average 55 x 5.

Conidia from 30 days' old potato plug:
3 septate 34–42 x 4–5, average 38 x 4.5.
4 septate, 38–55 x 4.5–5, average 48 x 4.5.
5 septate, 46–59 x 4.5, average 53 x 4.5.
Conidia:
From 45 days' old 4 per cent potato agar plus 1½ per cent dextrose:
All were 3 septate, measuring 29–41 x 4–5, average 35 x 4.5.
From 45 days' old beef agar (plus 10) plus ½ per cent dextrose—
1 septate, 17–27 x 4–5, average 23 x 4.5.
3 septate, 25–32 x 4–5, average 29 x 4.5.
Conidia from peach bud:
3 septate, 38–46 x 5–5.5, average 42 x 5.
5 septate, 46–59 x 5–6, average 55 x 5.5.

As pointed out by Sherbakoff [3] there is little profit in growing species of Fusarium on a wide variety of media. Characteristic growth on some of the more commonly used media was as follows:

On corn-meal agar: Hyphal growth colorless and scant, almost invisible except for white down near the upper margin of the slant. In seven days the slant was covered with colorless to pale salmon conidial masses of indefinite shape and size, often becoming a slime or pseudopionnotes.

On corn meal: Growth rapid, the white abundant hyphae covering the entire surface of medium (50 cc. in an Erlenmeyer flask of 100 cc. capacity) in 3 days and producing sporodochia more or less scattered over medium. Spore masses bright salmon. Drops of water 2 mm. or less in diameter appeared over surface. After 10 days, the spore masses were indeterminate masses of slime covering most of the surface. The mycelial mass was still white but there were present numerous dirty white to yellow "concretions" or sclerotia. In 20 days the surface was wrinkled and yellow. Aerial hyphae were white but the surface was nearly covered by aggregations of bead-like bright salmon-colored conidial masses.

On potato plugs: Aerial hyphae white and cottony; surface of plug, rty white; sclerotia numerous, cream-colored, 1–5 mm. in diameter. onidial masses salmon-colored, 4–5 mm. in diameter and composed of om 3 to several hundred smaller bead-like spore-masses averaging 5 mm. diameter, a few being 1 mm. but many less than .5 mm. in diameter.

On 4 per cent potato agar plus ½ per cent dextrose: In one week the owy white cottony aerial hyphae covered the tube-slant. At center, ile salmon-colored sporodochia ranging in diameter up to 1 mm. were ggregated to form a clump 7 mm. across.

On oatmeal paste: Sclerotia appeared in 10 days at margins of the edia. Conidial masses were salmon-colored and indefinite (pseudo-onnotes). Hyphae were cottony when young, dirty white when older.

On oatmeal agar: As on potato agar, but with scant production of onidia.

On steamed rice: White, cottony aerial hyphae, those at the surface medium, yellowish white. Conidial masses salmon-colored, usually rmless and slimy (pseudopionnotes), but occasionally there were deep-lmon sporodochia, which after 18 days' growth in mass had a delicate ddish color. No characteristic odor was present.

On 3 per cent prune agar: About the same as on cornmeal agar. onidial masses slimy but not so diffuse as on cornmeal agar.

On 3 per cent apple agar: White scanty aerial hyphae in loose wefts. edia turned from light brown to black in 4 days. No fruiting bodies.

[3] SHERBAKOFF, C. D. FUSARIA OF POTATOES. N. Y. Cornell Agr. Exp. Sta. Mem. 6, p. 87–270, 51 fig., ol. pl. 1915. Literature cited, p. 269–270.

On 2 per cent glycerin agar: In every particular resembled very closely growth on cornmeal agar.

Effect of temperatures on growth: On cornmeal growth was more rapid at 25° C. than at 7° C. but at the latter temperature the surface of the media (10 cc. in a 100 cc. Erlenmeyer flask) was covered with growth and conidial production was abundant in one week; germination was prompt at 7° C.

On August 27, 1920, twigs were taken from healthy peach trees and the leaves were removed to expose the newly formed buds. Part of these twigs were then sprayed with water containing conidia from pure cultures. The inoculated twigs were placed under a bell jar and others sprayed with sterile water were placed under a separate bell jar and regarded as checks or controls. In 10 days the fungus had invaded the buds, leaf scars and the cut upper end of the inoculated twigs forming white sporodochia on their surfaces. The leaf and blossom buds of the inoculated twigs were killed, whereas those of the checks remained healthy and after 26 days came out into leaf. The fungus was reisolated from the killed buds. Using the methods outlined above, inoculations were made after the leaves had fallen naturally on October 12, and on November 19, 1920, also on February 7, March 1, March 11, buds showing pink, and March 16, blossoms out, 1921. In all cases the inoculations were successful, and eventually the fungus could be made to fruit on the killed buds by placing them under conditions of sufficient moisture. In each experiment the fungus was reisolated from the inoculated buds.

The buds or blossoms subjected to inoculation and those used as controls were cut open and examined with the following results:

Experiment of Oct. 12, 62.5 per cent of buds on inoculated peach twigs were dead, control buds all alive.

Experiment of Nov. 19, 53 per cent of buds on inoculated peach twigs were dead, control buds all alive.

Experiment of Feb. 7, 98 per cent of buds on inoculated peach twigs were dead, control buds all alive.

Experiment of Mar. 1, 49 per cent of buds on inoculated peach twigs were dead, 3 per cent of control buds were dead.

Experiment of Mar. 11, 75 per cent of buds on inoculated peach twigs were dead, 9 per cent of control buds were dead.

Experiment of Mar. 16, 100 per cent of blossoms on inoculated peach twigs were dead, control blossoms all alive.

On February 19, Elberta and Champion nursery peach trees, growing in pots in the greenhouse, were sprayed with a suspension of conidia in water; bell jars were placed over the trees for four days. The half-opened blossoms were killed, as were most of the flower buds, and the fungus was fruiting on their surfaces.

Inoculations of both sweet and sour cherry blossom-buds were made on November 19 and March 11. Twigs were removed from the trees, sprayed with a suspension of spores in water and placed under bell jars. Other twigs sprayed with sterile water only were placed under bell jars and regarded as checks. The buds used on March 11 were much swollen, showing green at the tips. The results after one week were as follows:

Sweet Cherry:

Nov. 19, 100 per cent of buds on inoculated twigs were dead. Control buds were all alive.

Mar. 11, 72 per cent of buds on inoculated twigs were dead; 9% of the control buds were dead.

Sour Cherry:

Nov. 19, 31 per cent of buds on inoculated twigs were dead. Control buds were all alive.

March 11, 19 per cent of buds on inoculated twigs were dead; 4 per cent of the control buds were dead.

It is shown by these results that blossom-buds of the peach, sour cherry, and sweet cherry can be attacked and killed by the Fusarium at almost any stage of their development even to and at least partially including blossoming time, provided favorable conditions of temperature and moisture are present.

In all the experiments, infection occurred at the tip of the bud and developed very rapidly, 4 to 5 days usually being sufficient for infection and subsequent death of the bud. The killed buds were always black and watery within. Sweet cherry buds appear to be much more susceptible than those of sour cherry and somewhat more so than those of peach.

Aderhold, using blossom-buds of the sour cherry in April and May, obtained positive results from his inoculation experiments. His work was done indoors and the inoculated material was kept in moist chambers. Infection took place through epidermal cells of the blossom parts and the incubation period was 4 to 6 days in length. He states that infection takes place only under moist conditions and shows that in the years in which the disease was prevalent the spring rainfall was excessive.

Data as to the amount of damage caused by this disease are very limited. During seasons of heavy rainfall it is possible that damage often assigned to other causes may in part, at least, be due to this disease. During some seasons there is a high mortality of peach buds following a winter apparently free from temperatures low enough to kill them and when all other conditions appear to be favorable. In such cases as these, seemingly unexplainable, it is possible that *Fusarium gemmiperda* may be involved. Its distribution and the amount of damage caused by it are, however, unknown.

SUMMARY

A species of Fusarium apparently identical with *Fusarium gemmiperda* Aderhold was isolated from dead peach buds from Georgia.

A description of the fungus and its reaction to culture media are given.

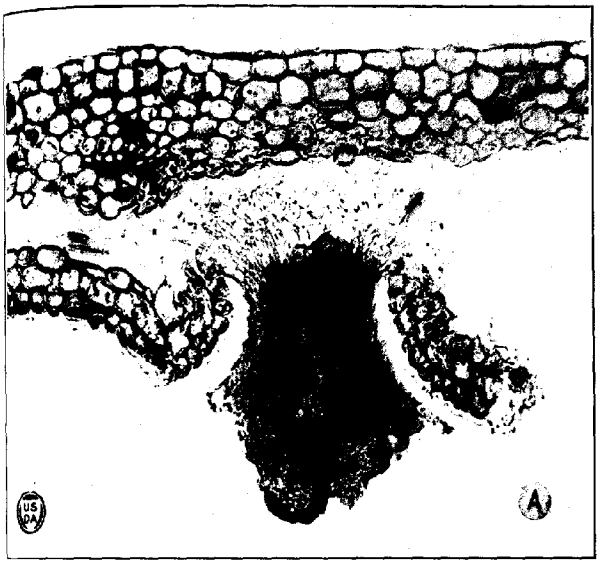
It is shown by experiment that under conditions of extreme moisture the fungus causes a budrot of the peach, sweet cherry, and sour cherry.

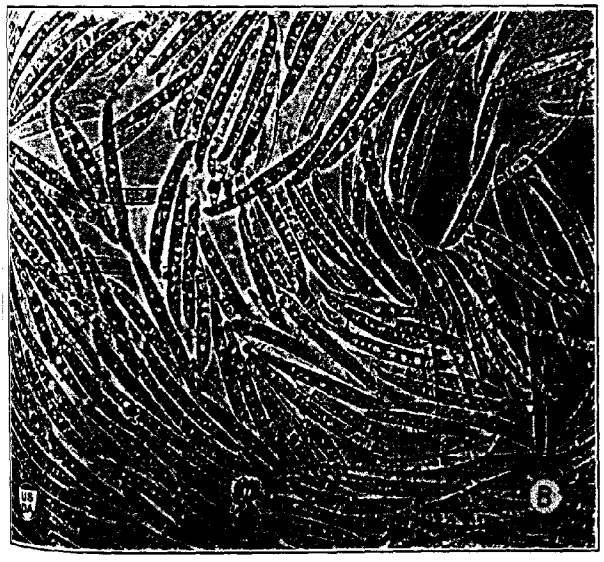
The disease is probably not of great importance under ordinary conditions, but it is possible that it may cause considerable damage during excessively moist weather. Its distribution is not known.

PLATE 1

A.—Photomicrograph of a section through two bud scales of a diseased peach bud showing a sporodochium of *Fusarium gemmiperda*.

B.—Photomicrograph of conidia of *Fusarium gemmiperda* from an 18-day-old culture on corn-meal agar.


A


B